2011

Taxonomy, Phylogeny, and Biogeography of the Net-winged Midges of Madagascar (Diptera: Blephariceridae: Paulianina and Eupaulianina)

Rebecca B. Sam
Iowa State University

Taxonomy, phylogeny, and biogeography of the net-winged midges of Madagascar (Diptera: Blephariceridae: *Paulianina* and *Eupaulianina*)

by

Rebecca B. Sam

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-Majors: Entomology; Ecology and Evolutionary Biology

Program of Study Committee:

Gregory W. Courtney, Major Professor

Lynn Clark

Matthew O'Neal

Marlin Rice

Timothy Stewart

Iowa State University

Ames, Iowa

2011

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDIX

ABSTRACT

Net-winged midges (Blephariceridae) are a group of lower Diptera uniquely adapted for life in torrential streams. Within this family, the subfamily Edwardsininae is restricted to south temperate regions — eastern Australia, southern South America, and Madagascar — whereas the Blepharicerinae occurs in both hemispheres. The Malagasy fauna contains both subfamilies, with the Blepharicerinae represented by an undescribed member of the tribe Apistomyiini. The remaining species belong to the Edwardsinine genus *Paulianina*, which currently is subdivided into the subgenera *Paulianina* and *Eupaulianina*. All members of the Malagasy fauna are endemic to the island country, where their habitat is endangered due to massive deforestation. Recent examination of historical material and collection of new specimens has required that the group be fully revised. My objectives included examining the diversity of net-winged midges on the island, testing the monophyly of all Edwardsininae and various Malagasy subgroups (genera and subgenera), as well as reconstructing the evolutionary relationships of species within *Paulianina* and *Eupaulianina*.

A complete revision of the Malagasy genera *Paulianina* and *Eupaulianina* is included. Keys to adult males, pupae and larvae are provided. Both morphological and molecular characters are used to investigate relationships within the group. Molecular characters were based on a single nuclear gene and single mitochondrial gene. Cladistic analyses, using both maximum parsimony and Bayesian inference, of both data sets support monophyly of the subfamily Edwardsininae and the genus *Paulianina*. Relationships within *Eupaulianina* remain less resolved, due partly to lack of associated adult material for several species. This research increases the number of described species from eight to twenty-seven. Phylogenetic analysis provides justification elevating the subgenera *Paulianina* sensu stricto

and *Eupaulianina* to generic status, while partially resolving relationships within each genus. Overall, this research provides the most thorough examination of the group to date.

I also provide an overview of the natural history of Malagasy net-winged midges and discuss primary threats to the survival of these unusual flies. Insects play a key role in aquatic ecosystems, however, little is known regarding Madagascar's aquatic insect diversity. Limited previous research suggests remarkably high levels of endemism. Ongoing, rampant deforestation is rapidly decreasing viable habitat across the island, such that several species have likely already gone extinct. Future research on these diverse and potentially sensitive bioindicators is imperative if we are to effectively preserve and manage these ecosystems in the future.

Keywords: P*aulianina*, *Eupaulianina*, Blephariceridae, Madagascar, net-winged midges, Gondwana, biodiversity

CHAPTER 1: GENERAL INTRODUCTION

Blephariceridae is a monophyletic group within the nematocerous Diptera. The net-winged midges, named for the secondary folding of their wings, are found on all continents excluding Antarctica, and on several continental islands (e.g., Madagascar, New Zealand, Sri Lanka). The lifespan of a blephariceridae is spent mostly in the larval form with pupation occurring in the larval habitat. Both larvae and pupae inhabit clear, well oxygenated, torrenticolous streams of mountainous regions, which may have been partially responsible for their lack of discovery until the 1840s.

Blephariceridae larvae and pupae are highly specialized to survive in torrential aquatic habitats. The larvae of most species are somewhat flattened and streamlined with the head, thorax, and first abdominal segment fused into a cephalic division, and with six ventral suctorial discs that permit attachment to the substrate in fast moving waters (Courtney 2000). Larvae are grazers, feeding mostly on diatoms found on submerged rocks (Alverson et al. 2001; Alverson and Courtney 2002). Pupae are dorsoventrally compressed, streamlined and firmly attached to rocks by three or four pairs of ventrolateral adhesive discs (Courtney 2003). Pupae tend to position themselves with the anterior end facing downstream to increase oxygen delivery across the respiratory organs (Pommen and Craig 1995). Adult blephariceridae superficially resemble crane flies (Tipulidae) and show a diversity of habits. The females of some species are predators of insects; however, many are nectarivorous or non-feeding. In most species, adults are short-lived and rarely venture beyond the riparian zone. These flies are an important, but underappreciated component of many stream ecosystems, where they may contribute significantly to secondary production (Anderson 1992) and/or represent an important food resource for fish (Courtney and Duffield, 2000).

Because the immature stages of net-winged midges typically inhabit clean, cool, well-oxygenated streams, these flies can be useful bioindicators of water quality (Lenat 1993).

Phylogenetic studies of the lower Diptera have placed Blephariceridae within the Blephariceromorpha, with Nymphomyiidae as the sister group to the Blephariceridae + Deuteroplebiidae (Wood and Borkent 1989; Courtney 1990, 1991; Oosterbroek and Courtney 1995). Analysis of pupal spiracular gill structure (Arens 1995) further supports this relationship. However, a recent molecular phylogenetic analysis using four nuclear genes places Blephariceridae in the Psychodomorpha along with Tanyderidae and Psychodidae (Bertone et al. 2008).

Alexander (1958) recognized four subfamilies within the Blephariceridae, as follows: Blephariceromorphinae, Edwardsininae, Apistomyiinae, and Paltostominae. However others (Courtney 2000; Zwick 1977) asserted that there are only two subfamilies, Blephariceromorphinae and Edwardsininae, with apistomyiines and paltostomines placed as tribes within Blephariceromorphinae. Differences in classification reflect partly the uncertainty about monophyly of the latter two clades and their relationship with other blephariceroids.

Malagasy net-winged midges were first described by Paulian (1949). Alexander (1952) described a single female adult as a new genus and species, *Paulianina hova*, which was placed in the subfamily Edwardsiniinae. Paulian (1953) later collected six larval morphotypes within *Paulianina*, and an unusual larva of the subfamily Blephariceromorphinae (tribe Apistomyiini). The latter remains undescribed. A comprehensive review by Stuckenberg (1958) described six new species, confirmed the placement of *Paulianina* in the subfamily Edwardsininae and proposed that the species of *Paulianina* be segregated into two subgenera, *Paulianina* sensu stricto (type species: *Paulianina hova* Alexander) and

Eupaulianina (type species: *Paulianina pamela* Stuckenberg). It seemed clear from morphological evidence that *Eupaulianina* was monophyletic (Stuckenberg 1958; Zwick 1977; Arens 1998), however questions remained regarding the monoplyly of *Paulianina*. Recent molecular and morphological analyses have shown both *Paulianina* and *Eupaulianina* to be monophyletic.

The current research uses both morphological and molecular data to classify species within *Paulianina* and *Eupaulianina*. The biological species concept states that species are defined by their capability to breed and produce viable offspring, requiring reproductive isolation of some form between species, be it sympatric or allopatric (DeQueiroz 2007). Net-winged midges are often sympatric but it is very difficult to determine reproductive isolation between species. However, in lieu of definitive determination of reproductive isolation, consistent morphological characters are used to define and place species within each genus. These results are combined with a phylogenetic analysis incorportating both morphological and molecular characters. Therefore, ideas from the phylogenetic species concept are also considered, where individual species are defined based on clade support (DeQueiroz 2007).

This study is the most comprehensive investigation of Malagasy blephariceridsince Stuckenberg's (1958). The current study includes a revision of the group with descriptions for all known species. Keys are provided for larvae, pupae and adult males. A phylogenetic analysis is included to illustrate relationships within each genus, and biogeographical patterns are discussed for both genera.

Thesis organization. This thesis is organized into three chapters. Chapter 1 summarizes the current knowledge of Malagasy blephariceridsids. Chapter 2, which will be submitted to *Zootaxa* for publication, contains a revision of the two genera *Paulianina*

Alexander and *Eupaulianina* Stuckenberg, including an examination of the phylogenetic relationships and biogeography the fauna. Chapter 3 provides a general conclusion.

As per Article 82 of the International Code of Zoological Nomenclature (1999) this document is not issued for the permanent scientific record or for the purpose of zoological nomenclature. Consequently, any species names contained herein should not be considered as published (*sensu* ICZN).

Original Classification of Edwardsininae (Blephariceridae)

Edwardsininae Edwards, 1929

Edwardsina Alexander, 1920

Subgenus ***Tonnoirina*** Edwards, 1929

tasmaniensis group Zwick, 1977

tasmaniensis subgroup Zwick, 1977

ferruginea subgroup Zwick, 1977

gigantea subgroup Zwick, 1977

torrentium subgroup Zwick, 1977

Subgenus ***Edwardsina*** Edwards 1929

Paulianina Alexander 1952

Subgenus ***Paulianina sensu stricto*** Stuckenberg 1959

hova Alexander, 1952, Madagascar

ingens Stuckenberg, 1959, Madagascar

umbra Stuckenberg, 1959, Madagascar

Subgenus ***Eupaulianina*** Stuckenberg, 1959

alexanderi Stuckenberg, 1959, Madagascar

pamela Stuckenberg, 1959, Madagascar

rivalis Stuckenberg, 1959, Madagascar

robinsoni Alexander, 1956, Madagascar

silva group, Stuckenberg, 1959

silva Stuckenberg, 1959, Madagascar

species H Stuckenberg, 1959, Madagascar

species I Stuckenberg, 1959, Madagascar

LITERATURE CITED

Alexander, C. P. 1952. Undescribed species of Nematocerous Diptera. Part III. *Bulletin of the Brooklyn Entomological Society* **XLVIII (4)**: 97-103.

Alexander, C. P. 1958. Geographical distribution of the net-winged midges (Blephariceridae, Diptera). *Proceedings of the 10th International Congress of Entomology* **1**: 813-828.

Alverson, A. J. and G. W. Courtney. 2002. Temporal patterns of diatom ingestion by larval net-winged midges (Diptera: Blephariceridae: *Blepharicera*). *Freshwater Biology* **47**: 2087-2097.

Alverson, A. J., G. W. Courtney and M. R. Luttenton. 2001. Niche overlap of sympatric *Blepharicera* larvae (Diptera: Blephariceridae) from the southern Appalachian Mountains. *Journal of the North American Benthological Society* **20**: 564-581.

Anderson, N. H. 1992. Influence of disturbance on insect communities in Pacific Northwest streams. *Hydrobiolobia* **248**: 79-82.

Arens, W. 1995. Structure and evolution of spiracular gills in pupae of net-winged midges (Nematocera; Blephariceridae). Part I. *Paulianina* and *Edwardsina* (subfamily Edwardsininae). *Canadian Journal of Zoology* **73**: 2318-2342.

Arens, W. 1998. Structure and evolution of spiracular gills in pupae of net-winged midges (Nematocera; Blephariceridae): Part III. Gill diversity in *Paulianina* (subfamily Edwardsininae). *Annals of Natal Museum* **39**: 83-114.

Bertone, M. A., G. W. Courtney and B. M. Wiegmann. 2008. Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. *Systematic Entomology* **33**: 668 – 687.

Courtney, G. W. 1990. Cuticular morphology of larval mountain midges (Diptera: Deuterophlebiidae): implication for the phlyogenetic relationships of Nematocera. *Canadian Journal of Zoology* **68**: 556-578.

Courtney, G. W. 1991. Phylogenetic analysis of the Blephariceromorpha, with special reference to mountain midges (Diptera: Deuterophlebiidae). *Systematic Entomology* **16**: 137-172.

Courtney, G. W. 2000. Family Blephariceridae. In *Contributions to a Manual of Palearctic Diptera. Appendix*. Papp, L. and Darvas, B. (eds.). Budapest: Science Herald. pp 7-30.

Courtney, G. W. **2003**. Blephariceridae, Net-Winged Midges. In *The Natural History of Madagascar*. Goodman, S.M. and Benstead, J.P. (eds.). Chicago: University of Chicago Press. pp 702-708.

Courtney, G. W. and R. M. Duffield. 2000. Net-winged midges (Diptera: Blephariceridae): A food resource for brook trout in montane streams. *Pan-Pacific Entomologist* **76**: 87-94.

International Commission on Zoological Nomenclature, 1999. *International Code of Zoological Nomenclature. 4th edition*. International Trust for Zoological Nomenclature, c/o Natural History Museum, London.

Lenat, D. R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water quality ratings. *Journal of the North American Benthological Society* **12**: 279-290.

Oosterbroek, P. and G. W. Courtney. 1995. Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society* **115**: 267-311.

Paulian, R. 1949. Sur la faune des cascades a Nosy Be. Le *Naturaliste Malgache* **1**: 31-32.

Paulian, R. 1953. Faune des eaux douces de Madagascar. II. Larves de Blepharoceridae [Dipt.]. *Memoires de L'institut Scientifuque de Madagascar* Serie E, Tome **IV**: 431-440.

Pommen, G. D. W. and D. A. Craig. 1995. Flow patterns around gills of pupal net-winged midges (Diptera: Blephariceridae): possible implications for respiration. *Canadian Journal of Zoology* **73**: 373-382.

Stuckenberg, B. R. 1958. Taxonomic and morphological studies on the genus *Paulianina* Alexander (Diptera: Belpharoceridae). *Memoires de L'Institue Scientifique de Madagascar* **10**: 97-198.

Wood, D. M. and A. Borkent. 1989. Phylogeny and classification of the Nematocera. In *Manual of Nearctic Diptera, Vol. 3*. McAlpine, J.F. and Wood, D.M. (coordinators). Agricultural Canada Monograph 32: 1333-1370.

Zwick, P. 1977. Australian Blephariceridae (Diptera). *Australian Journal of Zoology Supplement* **46**: 1-121.

CHAPTER 2: REVISION OF THE NET-WINGED MIDGES OF MADAGASCAR (DIPTERA: BLEPHARICERIDAE: *PAULIANINA* ALEXANDER AND *EUPAULIANINA* (STUCKENBERG))

An article to be submitted to *Zootaxa*
Rebecca B. Sam and Gregory W. Courtney

ABSTRACT

Net-winged midges (Blephariceridae) are a group of lower Diptera that are uniquely adapted for life in torrential streams. Within this family, the subfamily Edwardsininae is restricted to south temperate regions - eastern Australia, southern South America, and Madagascar - whereas the Blepharicerinae occurs in both hemispheres. The Malagasy fauna contains both subfamilies, with the Blepharicerinae represented by an undescribed member of the tribe Apistomyiini. The remaining species belong to the Edwardsinine genus *Paulianina*, which currently is subdivided into the subgenera *Paulianina* and *Eupaulianina*. All members of the Malagasy fauna are endemic to the island country, where their habitat is endangered due to massive deforestation. Recent examination of historical material and collection of new specimens has required that the group be fully revised. Our objectives included testing the monophyly of all Edwardsininae and various Malagasy subgroups (genera and subgenera), as well as reconstructing the evolutionary relationships of species within *Paulianina*.

A complete revision of the Malagasy genera *Paulianina* and *Eupaulianina* is included. Keys to adult males, pupae and larvae are provided. Both morphological and

molecular characters are used to investigate relationships within the group. Molecular characters were based on a single nuclear gene and single mitochondrial gene. Cladistic analyses, using both maximum parsimony and Bayesian inference, of both data sets support monophyly of the subfamily Edwardsininae and the genus *Paulianina s. l*. Relationships within *Eupaulianina* remain less resolved, due partly to lack of associated adult material for several species. This research increases the number of described species from eight to twenty-seven. Phylogenetic analysis provides justification elevating the subgenera *Paulianina sensu stricto* and *Eupaulianina* to generic status, while partially resolving relationships within each genus. Overall, this research provides the most through examination of the group to date.

We also provide an overview of the natural history of Malagasy net-winged midges and discuss primary threats to the survival of these unusual flies. Insects play a key role in aquatic ecosystems, however, little is known regarding Madagascar's aquatic insect diversity. Limited previous research suggests remarkably high levels of endemism. Ongoing, rampant, deforestation is rapidly decreasing viable habitat across the island, such that several species have likely already gone extinct. Future research on these diverse and potentially sensitive bioindicators is imperative if we are to effectively preserve and manage these ecosystems in the future.

Keywords: P*aulianina*, *Eupaulianina*, Blephariceridae, Madagascar, net-winged midges, Gondwana, biodiversity

INTRODUCTION

Blephariceridae, or net-winged midges, are a monophyletic group within the Lower Diptera that most likely originated in the Jurassic period (Courtney 2000a). These flies, named for the secondary folding of their wings, are a cosmopolitan group, found on all continents excluding Antarctica, and on several continental islands (e.g., Madagascar, New Zealand, Sri Lanka). The lifespan of a blephariceridae is spent mostly in the larval form, with pupation occurring in the larval habitat. Both larvae and pupae are extremely specialized, and inhabit only clear, well oxygenated, torrenticolous streams of mountainous regions, which may have been partially responsible for their lack of discovery until the 1840s. Most knowledge regarding the life history of the group has been based on larval and pupal stages because these stages are the most often collected.

Alexander (1958) asserted that the worldwide distribution of blephariceridae and their specialized larval structure indicate an old family perhaps dating as far back as the Mesozoic period. However, fossil data remain quite limited. This could be due to the fact that no life stage ventures far from its lotic habitat, providing little opportunity for fossilization to occur. Two fossils unearthed in the Magadan Region of Russia are dated to the Upper Cretaceous (94mya) (Lukashevich and Shcherbakov 1997) while two Burmese amber fossils date back to 100mya (Grimaldi et al. 2002). Recent discovery of two fossils representing two new genera from Inner Mongolia, China are dated between the Bajocian and Aptian eras, placing their age between 112-171mya (Zhang and Lukaschevich 2007).

Phylogenetic studies of the Lower Diptera have placed Blephariceridae within the Blephariceromorpha, with Nymphomyiidae as the sister group to the Blephariceridae +

Deuteroplebiidae (Wood and Borkent 1989; Courtney 1990, 1991; Oosterbroek and Courtney 1995). Analysis of pupal spiracular gill structure (Arens 1995) further supports monophyly of the Blephariceridae + Deuteroplebiidae. However, a recent molecular phylogenetic analysis using four nuclear genes places Blephariceridae in the Psychodomorpha along with Tanyderidae and Psychodidae (Bertone et al. 2008; Wiegmann et al. 2011).

Alexander (1958) recognized four subfamilies within the Blephariceridae: Edwardsininae, Blephariceinae, Apistomyiinae, and Paltostominae. However, others (Zwick 1977; Courtney 2000a) asserted that there are only two subfamilies, Edwardsininae and Blephariceinae, with apistomyiines and paltostomines placed as tribes within Blephariceinae. Differences in classification reflect uncertainty about monophyly of the latter two tribes and their relationship with other blephariceines.

Blephariceids were first recorded from Madagascar by Paulian (1949), although the species remained unnamed. Based on this material, Alexander (1952) described a single female adult representing a new genus and species, *Paulianina hova* Alexander, which he placed in the subfamily Edwardsininae. Paulian (1953) described six larval morphotypes and one pupal morphotype, placing them in *Paulianina*. He also described one larva, which he placed in the tribe Apistomyiinae. Alexander (1956) described a second species, *P. robinsoni* Alexander, again using a female as the holotype. In studies conducted during 1955-1956, Stuckenberg found six new species of *Paulianina*, including larvae and pupae for all new species. He also found the immature stages of two additional unknown species. Stuckenberg (1958) confirmed the placement of *Paulianina* in the subfamily Edwardsininae and proposed that the species of *Paulianina* be segregated into two subgenera, *Paulianina s. s.* (type

species: *Paulianina hova*) and *Eupaulianina* (type species: *Paulianina pamela* Stuckenberg) both of which are restricted to Madagascar. While the monophyly of *Eupaulianina* seems to be firmly established (Stuckenberg 1958; Zwick 1977; Arens 1998), questions remain regarding the possible paraphyly of *Paulianina* s. s. (Arens 1998). Both Zwick (1977) and Arens (1998) noted striking similarities between the pupal gill structure of *Paulianina* s. s. and South American *Edwardsina* s. str., but examination of the character is limited by the availability of South American specimens. Current morphological and molecular analyses (Figs. 150-154) firmly support both *Paulianina* and *Eupaulianina* as monophyletic, and therefore easch is raised to the generic level.

In this study, both morphological and molecular data was used to classify species within *Paulianina* and *Eupaulianina*. The biological species concept states that species are defined by their capability to breed and produce viable offspring, requiring reproductive isolation of some form between species, be it sympatric or allopatric (DeQueiroz 2007). Net-winged midges are often sympatric but it is very difficult to determine reproductive isolation between species. However, in lieu of definitive determination of isolation, consistent morphological characters are used to define and place species within each genus. These results are combined with a phylogenetic analysis. Therefore, ideas from the phylogenetic species concept are also considered, where individual species are defined based on clade support (DeQueiroz 2007).

Additional questions pertained to the biogeographic origins of the Edwardsininae. The present distribution of *Edwardsina* includes Australia and southern South America, whereas *Paulianina* and *Eupaulianina* are endemic to Madagascar. Stuckenberg (1958) asserted that the morphological differences between the Australian and South American

species was too great for the distribution to be explained based on continental drift, and he concluded that Edwardsininae was of northern origin. Zwick (1977) agreed that while *Edwardsina* did not provide compelling evidence of a transantarctic distribution, the idea of a northern origin could not explain the restricted Neotropical range (southern Chile and Argentina). Arens (1995) agreed with Zwick and asserted that, due to their restricted habitat and the weak flying capacity of the adults, edwardsinine distribution must be due to vicariance rather than dispersal. Present consensus accepts that the subfamily Edwardsininae has a Gondwanan distribution (Zwick 1977; Zwick 1998; Courtney 2003).

Madagascar faces ongoing ecological devastation in the form of increased deforestation, mining and indiscriminate pesticide usage, resulting in massive erosion and stream degradation, which is rapidly decreasing viable habitat across the island. Due to this ecological degradation several species have likely already gone extinct. For example, the recent Ambotavy pipeline built between Andasibe (Toamasina) and the coastal city Toamasina (Toamasina) cuts directly through the Vohimana Reserve (Draper 2010), destroying the collection site of a newly discovered species, *E. fantsona*. Since *E. fantsona* has only been documented at this single site, it is now likely extinct. In addition, several species that were previously collected by Stuckenberg (1958) are also likely extinct, as their specific aquatic habitat has long been degraded due to deforestation and human settlement. These circumstances create an urgency in the scientific community to document the remaining biodiversity of the island, and to increase conservation efforts to maintain the few undisturbed natural areas of this biological hotspot.

Objectives of Research

1. To test the monophyly of the Edwardsininae and various subgroups (i.e, genera, subgenera, and species groups) through cladistic analysis of morphological and molecular characteristics.
2. To examine the biogeographic history of the Malagasy blephariceridsd.
3. To fully describe all known and previously undescribed species of *Paulianina s. l.*, in larval, pupal, and adult stages, where specimens are available.
5. To create dichotomous keys for 4th-instar larvae, pupae, and adults of *Paulianina s. l.*
7. To determine the phylogenetic relationship between species within *Paulianina s.l.* through cladistic analysis of morphological and molecular characteristics.
8. To examine the biogegraphic history of species within *Paulianina s. l.*

Materials. This study was based on examination of larvae, pupae and adults of all known species of Malagasy Edwardsininae, most on loan from the Natal Museum, Pietermaritzburg, South Africa (NMP), courtesy of Brian Stuckenberg. Other material from the United States National Museum, Smithsonian Institution, Washington, D.C. (USNM), courtesy of Wayne N. Mathis, and from Iowa State University (ISIC), was also examined. Specimens included material preserved in alcohol and pinned. Also available were specimens collected in Madagascar from 2004 to 2007 by R.B.S., the majority of which were larvae and pupae. Association of larvae and pupae was based partly on ontogenetic methods (Hogue and Bedoya-Ortiz 1989; Courtney 2000b), where the pharate individual is dissected from the earlier stage. In this case, pupal respiratory organs were dissected from mature 4th-stage

larvae. Association of pupae and adults also used this method, with the pharate adult dissected from the mature pupa.

Preparation. Adult specimens were either stored in ethanol, mounted on slides, or pinned after chemical drying using hexamethydisalizane (HMDS). Adult specimens collected before 2004 were stored in 70% ethanol while specimens collected 2004-present were stored in 95% ethanol. The genitalia of selected adults were dissected, cleared with 10% potassium hydroxide (KOH) solution and preserved in microvials of glycerin. Larvae and pupae collected prior to 2004 were stored in 70% ethanol with the remaining specimens stored in 95% ethanol. Specimens were examined using an Olympus SZX-12 dissecting microscope and a Nikon E-800 compound microscope. Drawings were rendered using a drawing tube on either scope. Light micrographs of both larvae and pupae were captured via a SPOT RT® digital camera. Serial photomicrographs were collapsed using Helicon Focus 3.10®. Specimens prepared for scanning electron microscopy were cleaned and sonicated (5-10 sec), transferred into 100% ethanol, followed by critical point drying and a gold-palladium sputter coating. Scanning electron micrographs were captured using a JEOL JSM 5800LV Scanning Electron Microscope in Iowa State University's Microscopy and NanoImaging Facility. Drawings were created using the Wacom Intuos 4 Professional Pen Tablet integrated with Adobe Illustrator CS3.

Terminology. Terms for structures are based primarily on Courtney (2000b). Dorsal paramere refers to Stuckenberg's "tegmen". Gonocoxal lobes refer to Stuckenberg's "dististyles". Gonocoxites refers to Stuckenberg's "basistyles". Ventral parameres refers to Stuckenberg's "parameres". Wing terminology follows Stuckenberg (1958) with "spur"

defined as the distal portion of R_{3+4} that extends past R_s. Pupal respiratory organ terminology is based primarily on Arens (1995, 1997, 1998). Lamellae are counted from the lateral edge, with the outermost lamella being number one. Terms for larval chaetotaxy follow Stuckenberg (1958), Hogue (1986), and Courtney (1990). Larval sensillar regions are designated as AD, AL, LP and PP, and dorsal plates as M, AM, PM, R, S and T (all *sensu* Stuckenberg 1958) (Fig. 133). Tubercle refers to any stout, conical structure on the larva. Sensilla/sensillum refers to any innervated sensory structure. Primary sensilla are present on first-stage larvae. Secondary sensilla are additional sensilla present in subsequent instars. Seta(e) is a general term referring to both innervated and non-innervated extensions of the cuticle. Macrotrichia and microtrichia refer to any non-innervated setose structures, according to size. In regards to nomenclature, “comb. nov.” refers to “combinatio nova” which is defined as a new combination of name and epiphet. Paulian (1953) designated described but unnamed larvae using alphabetical names: Species A-G. Stuckenberg (1958) continued this protocol with Species H and Species I. The current revision will continue this with Species J. Due to Madagscar gaining independence in 1960, some location names have been alters. The village formerly known as “Perinet”, located next to Mantadia National Park and Analamazoatra Special Reserve, is now known as Andasibe. Original locale data is presented in quotes. Consistency Index (CI) refers to the measures of how characters fit to the cladogram. Retention Index (RI) is the fraction of potential synapomorphies relized in the cladeogram. The term “bootstrap” represents the number of replicates that support the node within a phylogenetic tree. The term “posterior probablities” represents the percentage of trees that support that node during the analysis.

Descriptive Format. Diagnoses are provided for all species. Complete descriptions are provided for each new species and any life stage previously not described. Redescriptions are made for previously described species where new specimens are collected. If no new specimens were available, I refer to original descriptions and the most recent review (Stuckenberg 1958). Descriptions are placed in phyletic order according to the combined morphological and molecular Bayesian analysis (Fig. 154). Remaining named species not included in the analyses follow in alphabetical order. This is followed by any unnamed species, defined as any species with only one life stage, beginning with Species J. Larval "cranial width" is defined as the distance between antennal bases. Adult palpomere proportions represent the length of proximal to distal articles. Leg segment percentages are mean values for the segment length: total leg length. Keys are provided for 4th-stage larvae, pupae and adult males. A key for adult females is not provided, as consistent diagnostic characteristics were not available. Measurements are given in millimeters (mm), followed by ranges in brackets. Abbreviations for life stages are L = larva; P = pupa; Pex = pupal exuvia; A = adult. Descriptions based on larval specimens collected prior to 2004 take into account the probable loss of color due to long-term storage in 70% ethanol. Site descriptions are provided for recent collections. Records prior to 2004 lack coordinates therefore, label data provided in quotes when necessary. Abbreviations for labels include the following: AMSR, Ambohitantely Special Reserve; ANSR, Analamazaotra Special Reserve; ANP, Andringitra National Park; ANA, Anosibe an'Ala; IRSM, Institute de Rechercher Scientifique de Madagascar; ISM, Ile St. Marie; MNP, Mantadia National Park; NM, Natal Museum, Pietermaritzburg; RNP, Ranomafana National Park; VOH, Vohimana Reserve. Other abbreviations for label and locality data are: coll = collected by; km = kilometer; m = meter;

pt km = kilometer post or marker; nr = near; N = north; S = south; Mt = mountain; R = river; Ntl = National; RS = reserve; SR = Special Reserve.

Ecological Definitions. Larval habitat can be separated into four categories: (1) splash zone refers to rocks close to the stream or waterfall that do not receive direct contact from the continual flow of water, but remain permanently moistened due to periodical splashing; (2) madicolous habitat refers to rocks that have a steady, thin flow of water across their surface; (3) small streams are defined as streams having a depth less than two-third of a meter, and a width less than three meters; (4) large streams are defined as streams having a depth greater than two-third of a meter and a width greater than three meters.

Phylogenetic Methods. Three exemplars from *Paulianina* and eleven exemplars from *Eupaulianina* were scored for morphological characters to test monophyly of the Malagasy species and to investigate their relationships. Two exemplars from *Paulianina* and nine exemplars from *Eupaulianina* were scored for both morphological and molecular characters from two separate genes to test for monophyly of the Malagasy species and their relationships. Two exemplars from *Edwardsina*, *E. chilensis* from South America and *E. confusa* from Australia, were scored as outgroups in order to cover the full geographic range of the group across the southern hemisphere. All life stages were scored for morphological characters while only larvae were used in molecular analysis. Outgroup was scored as zero. All characters were unordered and not weighted. Two genes were sequenced and used for phylogenetic analysis. Big Zinc Finger 2 (BZF2) is a transcriptional regulator that produces proteins with zinc finger domains. It is rapidly evolving single copy gene developed by John

K. Moulton, University of Tennessee. ND2 is a strongly evolutionarily conserved mitochondrial gene.

Morphological Analysis. Morphological analyses were based on 20 characters, including features of larvae, pupae, and adults. A separate analysis was performed using only larval and pupal characteristics as these life stages hold the majority of characters. The resulting matrices were analyzed using maximum parsimony (MP) and maximum likelihood (ML) in PAUP 4.0b10 (Swofford 2002). Character support was analyzed using branch support generated by PAUP. A morphological matrix was developed using the same species as in the molecular analysis and was analyzed using Mr. Bayes 3.1 (Ronquist and Huelsenbeck 2003) for comparison purposes.

Molecular Analysis. Each gene was analyzed separately before doing a combined analysis. Total DNA was extracted using the DNeasy DNA Extraction Kit from specimens preserved in 95% ethanol. Polymerase Chain Reaction (PCR) amplification was performed using an Eppendorf Mastercycler Gradient. Conditions of polymerase chain reaction were 94°C for 2 minutes to denature; 5 cycles of: 94°C for 30s, 55°C for 20s, 72°C for 2min; 14 cycles of: 94°C for 30s, 50°C for 15s, 72°C for 2min; 32 cycles of: 94°C for 30s, 45°C for 10s, 72°C for 2min. Primer sequences used were: for ND2: F: GCTANTRGGTTCATACCC; R: ARTGGCTGAAGTTTAGGCGATA and BZF2: F: CCNTTYGTYTGYCARCATTGYGG; R: CCRTCNGCRAANGCYTTCCARCA. PCR products were gel purified using the Gel Extraction Kit. Alignment created using Clustal X 2.0.12 (Gibson et al. 2009) and optimized by eye using SeAl 2.0 (Rambaut 2007). Molecular analysis performed using Mr. Bayes 3.1 (Ronquist and Huelsenbeck 2003). MrModelTest

(Nylander 2004) was used to estimate the best fitting substitution model. A model based upon general time reversal with gamma distributed rate heterogeneity and a significant proportion of invariable sites (GTR + I + G) was chosen. The Bayesian analysis conditions for the molecular analysis were as follows: 5,000,000 generation, four Metropolis-coupled Markov chains, sampled every 2000 generations, the first 3750 generations used as burn-in. Trees were viewed using Fig Tree v1.3.1. Character support was analyzed using posterior probabilities generated in the Bayesian analysis.

Combined analysis. Morphological and molecular data for each gene were partitioned in a combined Bayesian analysis using Mr. Bayes 3.1 (Ronquist and Huelsenbeck 2003). The Bayesian analysis conditions for the molecular analysis were as follows: 1,000,000 generation, four Metropolis-coupled Markov chains, sampled every 1,000 generations, the first 1,500 generations used as burn-in. Trees were viewed using Fig Tree v1.3.1. Character support was analyzed using posterior probabilities generated in the Bayesian analysis.

Natural History

Located 400 km from the eastern coast of South Africa, Madagascar vibrantly illustrates the effects of geographic isolation. Separation from Africa 165 million years ago led to an explosion in biodiversity and to levels of endemism (Plants 89%; Mammals 93%; Birds 58%; Reptiles 96%; Amphibians 99%) virtually unmatched by any other biotic region (Goodman and Benstead 2003). Irwin et al (2003) describe Malagasy Diptera as “abundant, diverse, and acutely unknown”. Like many dipterans, net-winged midges (>300 spp.) display remarkable levels of species richness and endemism in Madagascar. Of known Malagasy species, all net-winged midges are endemic (Irwin et al 2003). Geographic isolation has

apparently allowed rapid radiation of blephariceridsinto two distinct subgenera, *Paulianina* s. s. and *Eupaulianina*. *Paulianina* s. s. superficially resembles *Edwardsina*, whereas *Eupaulianina* displays distinctive and unique chaetotaxy. Net-winged midges were thought to be restricted to the remainder of the island's eastern rainforest belt that runs the length of the island. However, recent collections have revealed them in a high altitude stream within the central highlands.

Both *Paulianina* and *Eupaulianina* usually occur in forested streams, with the only exception being high altitude streams (>1,522m) in ANP. Larvae can remain attached to the substrata in water velocity up to 300-450 cm/s (Pommen and Craig 1995). The larvae of most species are somewhat flattened and streamlined with the head, thorax, and first abdominal segment fused into a cephalic division, and with six ventral suctorial discs that act as hydraulic suckers, permitting attachment to the substrata in fast moving waters (Courtney 2000a). In most species the body shape causes water to flow ventrolaterally across filamentous gills located between major body divisions, thereby enhancing respiration (Craig 1990). Larvae are found on any rock surface that has a constant influx of water; on submerged rocks or bedrock with a constant flow of water over the surface, in the splash zone of waterfalls, and even on the damp rock walls of streamside caves. *Paulianina* and *Eupaulianina* larvae usually inhabit areas where the water depth does not exceed 2-5cm (Sam, unpublished data). Colonized substrates typically are free of moss and other noticeable algal growth. The larvae are not found in slow-moving waters, where such thick algal growths are more common. Larvae are grazers, using their mandibles to scrape periphyton (thin film of bacteria, algae, and diatoms) off rock surfaces. The maxillae are then used to sweep the displaced food into their mouth. The bulk of their diet is comprised of diatoms

(Alverson et al. 2001; Alverson and Courtney 2002). Larvae are generally sedentary, moving slowly forward as they graze. They detach and reattach each disc in a regular pattern to move across the rock. When disturbed, larvae will exhibit a sideways escape pattern (Frutiger 1998). When ready to pupate, larvae of most species move to an area of the rock that has less flow.

Pupae are dorsoventrally compressed, streamlined and firmly attached to rocks by three or four pairs of ventrolateral adhesive discs (Courtney 2003). Malagasy pupae are often collected from aggregations in either slower current velocity than larvae or in the splash zone of rocks rather than directly in the flow. Pupae tend to position themselves with their wider anterior end facing downstream to form a "bluff" body, creating twin vortices across their respiratory organs, enhancing delivery of oxygen (Pommen and Craig 1995). Emergence is quick, completed in three to five minutes, with the adult rising to the water surface in an air bubble (Alexander 1963). The wings of the developing imago are fully formed while still in the pupal case. This forces the wings to be compressed tightly within the pupa, creating a network of characteristic folds, which forms the basis for the name, "net-winged" midge.

Adult blephariceridsemerge from the pupal case with wings fully hardened and developed, allowing adults to fly immediately upon emergence. In most blephariceridspecies, the adults have long legs and antennae, a slender body, and generally resemble many other adults in the Lower Diptera. Adult net-winged midges are rarely collected far from their natal habitat. Little is known about the feeding ecology of most adult blephariceridsAlthough feeding has not been documented for adult *Paulianina* and *Eupaulianina*, mouthparts indicate that they have similar habits as other blephariceridsIn many species, females have mandibles and are predaceous on other insects. Males and females that lack

mandibles are thought to be non-feeding or nectivorous. Adults are thought to live only 1-2 weeks and often mate quickly, sometimes immediately, after emergence (Courtney 2000b). Adults are generally collected next to, or above, the stream from which they emerged. *Paulianina umbra* adults have been observed linked together in long chains among the trees that overhang the stream. Using their forelegs to attach to the adult in front of them, and their hind legs to attach to the adult behind them, they make long strings of adult flies. In many other species, adults remain suspended from the underside of leaves or wet surfaces, gripping the surface with their fore- and mid legs, leaving their hind legs to hang behind them in a knock-kneed fashion (Courtney 2000b). Adults fly with an erratic, circular "dancing" motion.

Although the overall ecology of the group remains poorly known, blephariceridsmight play an important role in the conservation of aquatic systems. Because the immature stages of net-winged midges typically inhabit clean, cool, well-oxygenated streams, these flies can be useful bioindicators of water quality (Lenat 1993; Courtney et al. 2008). Malagasy blephariceridsinhabit streams at all elevations, having been collected from streams as they enter the sea, to high altitude (1,725m above sea level) streams on Mount Andringitra and Mount d'Ambre. Temperatures of streams with *Paulianina* ranged from 18-22˚Celsius. Blephariceridshave long been considered a rare fly due to their extremely specific habitat requirements. However, when the habitat is suitable, they can be present in large numbers (Johns 1996; Courtney, unpublished data), and may contribute significant biomass to stream ecosystems (Anderson 1992). Furthermore, these flies can be an important source of food for local fish (Courtney and Duffield 2000).

Blephariceridsshow high levels of sympatry in Malagasy streams, with several species being collected from the same stream, and often from the same rock. When

Paulianina and *Eupaulianina* larvae are collected from the same rock, *Eupaulianina* specimens will greatly outnumber *Paulianina*.

Because collecting and observations were done sporadically over several years and different months, the phenological patterns of Malagasy blephariceridsare difficult to determine. Overall, collecting has been done in January, February, May, June, October, and December. However, limited time has been spent at each site, thereby precluding long-term observations of resident species. At many sites, multiple life stages of both genera can be found at different times of year, making it difficult to determine whether *Paulianina* and *Eupaulianina* are univoltine or multivoltine. Larvae have been collected year-round at many streams. However, adult collection has been much more limited. The fact that adults of *Paulianina umbra* have been collected from several locations in October, December, and January could indicate that *Paulianina* may be mutlivoltine, or simply asynchronous, as is common in tropical climates (Courtney 2003).

Subfamily **Edwardsininae** Edwards 1929

Larva: Small intercalary segments present between abdominal segments 3-7. Gills located on intercalary segments. Antennae 1-2 segmented. Pupa: Respiratory organs with 10+ distinct lamellae. Lamellae clearly separate. Cephalic ecdysial lines reduced. Adult: Head normally dichoptic; eyes not divided. Maxillary palpi 4-5 segmented. Wings with 10 veins reaching margin. M_3 present. Seminal vesicles supported by chitinous pillar established on ventral bridge. Front trochanters short, 0.5x length of coxae.

Genus ***Paulianina*** Alexander 1952

Paulianina Alexander 1952.

Syn: *Paulianina* subgenus *Paulianina* Stuckenberg 1958.

Type species: *Paulianina hova* Alexander 1952

Diagnosis.—Larva: Cranial sclerite complete . 4th instar antennae two-segmented, with basal segment twice as long as apical segment; 3rd instar antennae two-segmented, subequal; remaining instar antennae single segment. Maxillae very large and prominent. Distinct reticulation along proximal edge of maxillary palpus. Clypeolabrum with 5-6 prominent clypeolabral sensilla distributed vertically. Intercalary divisions complete, not narrowed medially. Anal segment, composed of abdominal segments 7-10, not clearly demarcated, appears tri-lobed, demarcated from abdominal segment 6 by dorsolateral suture. Outer lobes of anal segments function as prolegs. No dorsal sclerotization and little to no dorsal tubercles present. 4th- and third-stage larvae with two antennal segments, remaining instars with one antennal segment.

Pupa: Respiratory organs with 8-9 distinct lamellae. Lamellae clearly separate, except in *P. umbra*, in which the first lamella is modified into a transverse bulge. Papillae well developed, circular, usually lacking spinelets or projections.

Adult: Head normally dichoptic; eyes not divided; ommatidia subequal; 15 antennal segments. Mandibles absent (male), mandibles present or absent (female). Maxilary palps 4-segmented. Mesopleural fold simple, slightly curved. Anal vein complete; anal cell open. Radial spur distinct in wing. Apex of M_{1+2} strongly arched upwards. Tibial spurs 1,1,1. Ventral parameres may or may not be present, if present, encased in tubes. Ventral plate

poorly differentiated from the ventral bridge. Chitinous pillar connects ventral bridge to the apodeme.

Included Species.—*Paulianina* Alexander, 1952:

hova Alexander, 1952

ingens Stuckenberg, 1958

umbra Stuckenberg, 1958

lehibe, new species

ratsipika, new species

Species J

Genus ***Eupaulianina*** (Stuckenberg 1958)

Eupaulianina (Stuckenberg 1958) comb. nov.

Syn: *Paulianina* subgenus *Eupaulianina* Stuckenberg 1958.

Type species: *Eupaulianina pamela* (Stuckenberg) 1958.

Diagnosis.—Larva: cranial sclerite complete. 4th instar antennae two-segmented and subequal; remaining instars antennae single segmented. The E2 sensillum displays a reticular vertical pattern. Clypeolabrum with 6 prominent clypeolabral sensilla distributed horizontally. First intercalary segment completely divided, remaining intercalary divisions may or may not be narrowed along midline by the joining of median divisions. Anal division, composed of abdominal segments 7-10, not clearly demarcated from abdominal segment 6. Prolegs distinct from dorsal anal division (except in *E. vohimalama*). Abundant dorsal sclerotization and tubercles present. 4th stage larvae with two antennal segments, remaining stages with one antennal segment.

Pupa: Respiratory organs with 5-7 swellings rather then distinct respiratory lamellae, except in Sp. I, which displays a similar form to *P. umbra*. Lamellae adjacent, with little to no space between. Papillae well developed, circular with spinelets and/or projections.

Adult: Head normally dichoptic; eyes not divided; ommatidia subequal; fifteen antennal segments. Mandibles absent (male), mandibles present or absent (female). Maxilary palps 5-segmented. Mesopleural fold simple, slightly curved. Radial spur distinct in wing. Tibial spurs 1,0,1. Ventral parameres present. Ventral plate well differentiated from the ventral bridge. Lateral aedeagal filaments may enter ventral parameres. Chitinous pillar connects ventral bridge to the apodeme.

Included Species—*Eupaulianina* (Stuckenberg), 1958:

vohimalama, new species

alexanderi, (Stuckenberg) 1958, comb. nov.

silva (Stuckenberg) 1958, comb. nov.

telofantsy new species

tsilobe new species

fantsona new species

marobotsin new species

pamela (Stuckenberg) 1958, comb. nov.

rivalis (Stuckenberg) 1958, comb. nov.

tandroka new species

borivody new species

botsimpatsy new species

korontantsilo new species

marangitsilo new species

ratsilo new species

robinsoni (Alexander), 1956 comb. nov.

Species K

Species L

Species M

Species N

Species O

Table 1. Gill filaments and antennal segments of larval *Edwardsina*, *Paulianina* and *Eupaulianina*

Table 1

Instars	**1**		**2**		**3**		**4**	
	G.F.	**A.S.**	**G.F.**	**A.S.**	**G.F.**	**A.S.**	**G.F.**	**A.S.**
Edwardsina	0	1	1	1	3	1	5	1
Paulianina	0	1	1	1	3	2	5	2
Eupaulianina	0	1	1	1	3	1	5	2
Other genera	0	1	1	1,2	3,4-5	1,2	5,7-9	1,2,3

GF = Gill Filaments AS = Antennal Segments

Keys to Malagasy Blephariceridae

Instar IV

(unknown in *Paualianina hova* and *Eupaulianina robinsoni,* Species K, Species L, Species M, Species N, Species O, Species P)

1. Maxillae approximately ½ size of head capsule, body with or without dorsal sclerotizations or tubercles (Figs. 1-8, 47, 48): *Paulianina*.....….2

– Maxillae approximately 1/5 size of head capsule, prominent sclerotizations across body, numerous tubercles may be present (Figs. 9-24, 76, 86, 101): *Eupaulianina* ….....……5

2(1). Dorsum with tubercles or sensilla Figs. 2-7).....……...…….3

– Body devoid of tubercles (Fig. 1)……...……...*P. ingens* Stuckenberg

3(2). Four large spine-like sensilla arranged in a row across medial segments, with two large spines on intercalary segments (Fig. 6)…*P. lehibe* **sp. nov.** Sam & Courtney

– Dorsum with numerous minute dorsal sensilla (Figs. 2-5, 7)….....…....…..…….4

4(3). Dorsum of medial segments with two rows of minute acutiform sensilla across anterior and posterior borders, intercalary segments with single row of similar tubercles (Figs. 2-5)…….….....…….*P. umbra* Stuckenberg

– Dorsum of medial segments with two horizontal rows of short, stout, pointed, dark sensilla, anterior row with central sensilla clustered to appear as a long thin plate, posterior row remain separate, intercalary segments with sensilla similar to anterior row on medial segment (Fig. 7).....*P. ratsipika* **sp. nov.** Sam & Courtney

5(1). Dorsal plates cover, or almost completely cover each abdominal segments; plates covered with numerous, evenly spaced microsculpturing (Figs. 13, 24, 83, 84,

110, 111) …….....……..6

– Dorsal plates distinct but do not cover any abdominal segment; AM with or without spines or some form of tubercle-like structure…….....…….……..……7

6(5). Abdominal and anal plates covered with round, smooth, flat-topped, evenly dispersed microsculpturingt; AM lacking large spines (Figs. 13, 83, 84) .....…*E. rivalis* Stuckenberg

– Abdominal, intercalary segments, and anal division covered with dome-like, evenly dispersed microsculpturing; AM with two large cone-shaped areas tipped with stout conical sensilla (Figs. 24, 110, 111).....*E. tandroka* **sp. nov.** Sam

7(5). AM with three elongate, dark, spines set evenly on large ovoid plate (Figs 14, 15, 16, 10)…….....….8

– AM without elongate spines…….…..…13

8(7). Lateral spines of AM at least 2x greater in length than the central spine (Figs. 10, 14, 23, 63-65).....…9

– Lateral and central spines of AM of approximately equal length….....….11

9(8) Spines of AM narrow, slender, tipped with slender pointed sensilla……...…...10

– Spines of AM stout, lateral spines strongly curved anteriorly or anterolaterally, tipped with stout pointed sensilla (Figs. 10, 63- 65).....…….*E. pamela* Stuckenberg

10(9) Lateral spines of AM proclinate lacking basal spines. (Figs. 14, 91) .....…*E. silva* Stuckenberg

– Lateral spines of AM reclinate with stout spine emerging from base of spine (Fig. 23)….....*E. ratsilo* **sp. nov.** Sam

11(10). Cranial sclerite produced posteromedially into a broad lobe; lobe reaches posteriorly between, and past, each AD plate (Figs. 15)...*E. tsilobe* **sp. nov.** Sam & Courtney

– Cranial sclerite without broad lobe (Figs. 16, 20, 100, 129)……...…12

12(11). AM with three narrow spine-like tubercles tipped with pointed sensilla, proclinate; no additional tubercles or sensilla (Figs. 16, 100).....…….....……*E. telofantsy* **sp. nov.** Sam & Courtney

– AM with three stout spine-like tubercles tipped with pointed sensilla, reclinate; two minute tubercles arranged marginally around lateral spines (Figs. 20, 129)……..…...*E. marangistilo* **sp. nov.** Sam

13(7). Dorsal plates rough, with conical tubercles (Figs. 9, 11, 17-19, 22, 61, 62, 67, 75, 119).....14

– Dorsal plates smooth, lacking tubercles…...*E. vohimalama* **sp. nov.** Sam & Courtney

14(13). Dorsal plates smooth, lacking microscupturing; (Figs. 11, 18, 19, 22, 75, 119).....….15

– Dorsal plates with evenly spaced microsculpturing (Figs. 9, 17, 61, 62, 67).....18

15(14). AM with several (i.e., more than three) rounded or conical tubercles (Figs. 18, 19, 119)…….....……16

– AM with three conical tubercles, but no additional tubercles (Figs. 11, 22, 75)..…….....……...17

16 (15). AM with three conical tubercles distributed evenly across plate, and an additional 4-6 smaller tubercles scattered randomly across plate (Fig. 18). .....*E. korontsilo* **sp. nov.** Sam & Courtney

– AM with approximately 7 rounded tubercles arranged in alternating pattern across plates (Figs. 19, 119)...……..…….*E. marobotsin* **sp. nov.** Sam

17(15). AM with three large conical tubercles, the largest in middle, each tipped with a setiform sensilla and with additional tubular processes attached at the base (Figs. 11, 75).....…….....…….*E. alexanderi* Stuckenberg

– AM with three piceous, small conical tubercles, without additional basal processes (Fig. 22).....*E. botsimpatsy* **sp. nov.** Sam

18 (14). Integument with scattered minute conical tubercles arranged horizontally from lateral margins of AM and PM; (Figs. 17)…*E. borivody* **sp. nov.** Sam & Courtney

– Integument lacking minute tubercles; integument rugose (Figs. 9, 61, 62, 67)..…….....…….*E. pamela* Stuckenberg

Pupae

(unknown in *P. hova*, *E. botsimpatsy*, *E. ratsilo*, *E. tandroka*, Species J)

1. Respiratory organ with 8 or more lamellae (Figs. 25, 26, 42, 44, 49, 54) *(Paulianina)* ….....2

– Respiratory organ with 7 or fewer lamella (*Eupaulianina*).....…….....…4

2(1). Respiratory organ with 9 distinct lamellae, without transverse bulge (Figs. 26, 42, 54)…….....……...3

– Respiratory organ with 8 distinct lamellae and a transverse bulge (Figs. 25, 44, 49).....…….....…..*P. umbra* Stuckenberg

3(2). Lamellae plate like, individual lamellae arranged parallel across plate (Figs. 26, 53).....*P. lehibe* **sp. nov.** Sam & Courtney

– Lamellae arranged longitudinally, with lamella 3 slightly curving towards the anterior ends of lamellae 4-6 which form a triangular cluster in the middle of the respiratory organ (Fig. 42).....*P. ingens* Stuckenberg

4(1). Respiratory organ comprised of 7 swellings (Figs. 34, 122).....*E. marobotsin* **sp. nov.** Sam

– Respiratory organ comprised of 6 or fewer lamellae or swellings.....5

5(4). Respiratory organ with 6 lamellae or swellings (Figs. 29, 30, 32, 93, 114, 116).....6

– Respiratory organ with five or fewer lamellae or swellings.....9

6(5). Some lamellae partially merged.....7

– Lamellae not merged, distinct from each other (Figs. 29, 93, 114).....8

7(6). Lamellae 1-2 partially merged. Lamellae five and six almost completely merged. Lamellae 2-5 separated by shallow gaps (Fig. 32, 116).....*E. borivody* **sp. nov.** Sam & Courtney

– 1st lamella large, swollen, pointed at outer apex; lamellae 4-6 appear as distinctly separated swellings; lamellae 2 and 3 merged, with small gap basally; lamellae 6 extended around base of lamellae 3-5, ending at groove separating lamellae 2 and 3 (Figs. 30, 94).....*E. tsilobe* **sp. nov.** Sam & Courtney

8(6). 1st lamella largest, 3x width of lamellae 2-6 combined; 6th lamellae extends around base of lamellae 3-5, with apex at the juncture of lamellae 2 and 3 (Figs. 29, 93).....*E. silva* Stuckenberg

– Lamellae one and six meet posteriolateraly, forming a basket-like structure. Lamellae four and five parallel to each other, curving toward posteriolateral margin of respiratory organ (Fig. 114).....*E. vohimalama* **sp. nov.** Sam & Courtney

9(5). Respiratory organ with 5 lamellae (Figs. 7, 27, 31, 35, 37, 38, 88, 103).....10

– Respiratory organ with fewer than 5 lamellae (Figs. 28, 33, 36, 39, 40, 79, 125, 130).....15

10(9). Lamellae separated by gaps approximately 0.5x the width of middle lamellae (Figs. 27, 37, 71).....11

– Lamellae compact, with narrow gaps between lamellae (Figs. 31, 35, 38, 88, 103).....12

11(10). Lamella 5 elongated, >2x as long as lamella 1, extending as a narrow process halfway across the posterior margin of respiratory organ. Lamellae 2-4 subequal in length (Figs. 27, 71).....*E. pamela* Stuckenberg

– Lamella 5 rounded, upright. Lamella 1 rectangular with point towards lateral margin of pupa; lamellae 2-4 rectangular, upright, with flattened tops (Fig. 37).....Species M

12(10). Lamellae present as simple smooth, rounded swellings, lacking raised areas in middle of lamellae (Figs. 31, 88, 103).....13

– Five lamellae present as swellings, raised centrally to give the appearance of a mound in the middle of each (Figs. 35, 38).....14

13(12). 1st lamella pointed laterally; 5th lamella with narrow lobe extending along posterior margin of lamellae 3 and 4 (Figs. 31,103).....*E. telofantsy* **sp. nov.** Sam

– 1st lamella rounded laterally; 5th lamella with broad lobe extending along posterior margin of 3rd and 4th lamellae (Fig. 88).....*E. rivalis* Stuckenberg

14(12). Pupa constricted at alar sclerite (Fig. 35).....Species K

– Pupa ovoid, not constricted at alar sclerite (Fig. 38).....Species N

15(9). Respiratory organ with 3 or 4 lamellae (Figs. 28, 36, 40, 79).....16

– Respiratory organ with lamellae merged into 1 broad lobe (Figs. 33, 39, 125).....18

16(15). Respiratory organ with 4 distinct lamellae, present as swellings with limited space between. (Fig. 40).....Species P

– Respiratory organ with 4 lamellae that are mostly fused (Figs. 28, 36, 79) 17

17(16). Respiratory organ nearly twice as wide as long; lamella 1 sharply rounded laterally; gap between last two lamellae transects most of respiratory organ (Fig. 36).....Species L

– Respiratory organ about as wide as long; lamella 1 broadly rounded laterally; gap between last two lamellae transects less than half of respiratory organ (Figs. 28, 79).....*E. alexanderi* Stuckenberg

18(15). Respiratory organ with small lobe at posteromedial margin. (Fig. 33).....*E. korontantsilo* **sp. nov.** Sam & Courtney

– Respiratory organ with large rounded lobe at posteromedial margin. (Fig. 39).....Species O

Adult Males

(unknown in *P. hova*, *P. lehibe*, *P. ratsipika*, *E. robinsoni*, *E. telofantsy*, *E. borivody*, *E. korontantsilo*, *E. marobotsin*, *E. marangatsilo*, *E. vohimalama*, *E. botsimpatsy*, *E. ratsilo*, *E. tandroka*, Species J, Species K, Species L, Species M, Species N, Species O, Species P)

1. Tibial spurs 1-1-1. Maxillary palpi 4-segmented *(Paulianina)*.....2

– Tibial spurs 1-0-1. Maxillary palpi 5-segmented (*Eupaulianina)*.....3

2(1). Tarsi conspicuously white for most of length; aedeagal filaments with slight inclination at apex (Fig. 140).....*P. umbra* Stuckenberg

– Tarsi not conspicuously white; aedeagal filaments with distinct hook at apex (Fig. 138).....*P. ingens* Stuckenberg

3(1). Aedeagal filaments lanceolate (Figs. 142, 145).....4

— Aedeagal filaments elongate and curved (Figs. 143, 146, 147, 149).....5

4(3). Ventral bridge with broad lateral extensions connecting to ventral plate, apodeme large with prominent ridge along medial line (Fig. 145) .....*E. rivalis* Stuckenberg

— Ventral bridge narrow, apodeme reduced with moderate ridge along medial line (Fig. 142).....*E. pamela* Stuckenberg

5(3). Three aedeagal filaments; apodeme well defined (Figs. 147-149).....6

— Two aedeagal filaments, medial filament absent; apodeme reduced, distal margin weakly defined (Fig. 143).....*E. alexanderi* Stuckenberg

6(5). Dorsal paramere with round lobes on lateral apices (Figs. 147-149).....7

— Dorsal paramere lobed along median line (Fig. 146) .....*E. silva* Stuckenburg

7(6). Ventral plate annular, surrounding the base of aedeagal filaments (Fig. 149) .....*E. tsilobe* **sp. nov.** Sam & Courtney

— Ventral plate shaped as half-circle with lobes at lateral apices (Fig. 147) .....*E. fantsona* **sp. nov.** Sam

Species Descriptions: *Paulianina* Alexander

Paulianina hova Alexander 1952

Paulianina hova Alexander 1952: 227-230. Stuckenberg 1958: 136-137.

Description based on and Alexander (1952) Stuckenberg (1958), from a single type specimen, adult female, collected at Mt. Tsaratanana in October 1949.

Diagnosis.— Large blephariceridid. M_4 and Cu_1 fused for short distance. Stellate groups of dark spicules on pleural membrane.

Description.— Larva: Unknown.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Size: large. Measurements (N= 1): Total length = 5.0mm; Wing length = 7.0mm; Wing width = 2.81mm.

Head: Labrum elongate, just longer than three basal flagellar segments, apical ridge serrated. Mandibles blunt. Palpomere proportions from base to apex: 34:28:8:11; second palpal segment enlarged apically. Antennal flagellomeres stout, becoming more slender apically. Ultimate flagellomere subequal [in length?] to pentultimate flagellomere. Chaetotaxy: occiput with scattered sparse setae; flagellomeres with fine microtrichia, short, inconspicuous macrotrichia on some flagellomeres.

Thorax: Spur 1.5x length of r-m; basal section of M_{3+4} in line with r-m; M_4 abruptly flexed on middle portion; M_4 and Cu_1 fused for short distance; macrotrichia almost absent on stigma. Last tarsomere 2x length of penultimate tarsomere. Claw with two large, two

medium, and one small tooth along underside; fine hair-like teeth at base. Chaetotaxy: Lateral setae of scutellum sparse.

Coloration: Pale on intersegmental membranes. Mesonotal prescutum with six dark stripes. Scutellum gray pruinose, posterior border dark. Abdominal tergites dark, ringed at idlength with orange yellow.

Terminalia: Eighth sternite with setae along posterior margin of median fold. Spermathecae subspherical, simple.

Type material.—Holotype [adult female]: MADAGASCAR. Mount Tsaratanana, 1500m, October 1949, coll. R. Paulian. Specimen dissected and slide-mounted [IRSM].

Paulianina ingens Stuckenberg 1958 (Figs. 1, 41-43, 138)

Paulianina ingens Stuckenberg 1958: 144-148. Zwick 1977: 21, 27 (review of morphology and phylogenetics). Arens 1995: 2326. Arens 1998: 83—90, 106, 108-109, 111-112

Diagnosis.— Large blephariceridae. Larva: Dorsum without large tubercles; may or may not have round prominences along midline of medial segments. Pupa: Respiratory organ with 9 distinct respiratory lamellae; tubercles round and wrinkled. Adult: Hypopharynx with distinct marginal serrations. Cu and M_4 not fused for any distance.

Description.—Larva (Figs. 141, 42): Measurements, instar III (n = 1): Total length 3.3mm, cranial width 0.4mm, basal antennal segment 0.1mm, apical antennal segment 0.12mm; instar IV (n = 17): Total length 8.9mm (7.9-10.1), cranial width 0.7mm (0.6-0.8), basal antennal segment 0.24mm (0.2-0.24), apical antennal segment 0.21mm (0.2-0.24); larva stout and lacking any tubercles or noticeable setae. Ecdysial line with little to no stem; posterior margin of frontoclypeal apodeme extended nearly to posterior cranial margin.

Coloration: Cranial sclerites dark with pale piebald sections laterally; remaining cephalothorax uniformly light brown. Medial and intercalary segments and prolegs uniformly light brown; round prominences may or may not be present along the dorsal midline of the abdominal segment of cephalothorax and each medial segment; prominence darker then surrounding integument. Anal division light brown, tri-lobed, not divided; outer lobes of anal division blunt, center lobe blunt, triangular. Chaetotaxy: Body scattered with slight, dark, acutiform sensilla; prolegs with dense apical patch of long, yellow setae.

Pupa (Figs. 41-43): Measurements (based on Stuckenberg 1958), male length 6.0mm; female length 6.62mm. Cuticle dark brown; body shape fusiform. Integument: Dorsal papillae present, uniformly distributed on anal tergite, abdominal segments, and metatergite; papillae ovoid with broadly wrinkled appearance; cuticle between papillae finely wrinkled. Respiratory organs elongated transversely. Respiratory organ with nine distinct lamellae. Lamellae arranged longitudinally, with lamella 3 slightly curving towards the anterior ends of lamellae 4-6 which form a triangular cluster in the middle of the respiratory organ.

Adult male (based on Stuckenberg 1958) (Fig. 138): Size: large.

Head: Hypopharynx with strong marginal serrations. Labrum elongate-triangular, length little less than basal two flagellar segments, tapering steadily to an acute point. Palpomere proportions from base to apex: 30:24.5:12:12.5. Antennal flagellomeres stout basally, progressively thinner apically; apical segment longer than penultimate; microtrichia fine, short; macrotrichia present on all segments. Chaetotaxy: Occipital setae abundant, long.

Thorax: Cu and M_4 not fused for any distance; Rs very short, ¼ length of R_4. Chaetotaxy: Scutellum with numerous, long setae laterally.

Terminalia: (Fig. 138) ventral epandrium with ≈ 15 prominent setae along posterior margin; gonostylus and gonocoxites setose; gonostylus simple; gonocoxites weakly sclerotized and not clearly demarcated from sternite 9; gonocoxal lobes simple, more slender than in *P. umbra* and extending in a more lateral direction than in *P. umbra*; aedeagal rods extend dorsally at an anterior angle, each with a distinct hook shape at apical end. Ventral parameres present, not closely associated with aedeagal rods; dorsal parameres broad, form a continuous hood over aedeagal rods; apodeme paddle-shaped, blunt anteriorly.

Type Material.—Holotype [adult female]: MADAGASCAR: Mt. Tsaratanana, 1949, coll. R. Paulian. [IRSM] **Other material examined**.—MADAGASCAR: *Fianarantsoa*: Antanifotsy, Andringitra National Park, Kimoro R on east slopes of Ambaravarandanitra Mt., 19 January 1958 [L]; Antanifotsy, Andringitra National Park, Jomando R., 10 January 1958 [L]. All specimens collected by B. Stuckenberg. Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January, 2007 [L]. All specimens collected by R. Sam.

Distribution.—*Paulianina ingens* is widely distributed across Madagascar, though not locally abundant. It is sympatric with *P. umbra* and most *Eupaulianina* species, and can be collected from the same rock with several other species. When collected among other species, *P. ingens* is usually less abundant than *P. umbra* and *Eupaulianina* species.

Bionomics.—Larvae and pupae can be collected year-round. The only adult female specimen was collected during the rainy season, in January. Larvae and pupae originally described by Stuckenberg were collected in larger streams, on more submerged rocks than *P. umbra*. However, this did not seem the case in the 2004-2007 collecting trips, during which larvae were collected in similar habitat as *P. umbra*.

Paulianina umbra Stuckenberg 1958 (Figs. 2-5, 25, 44-52, 136, 139-141)

Paulianina umbra Stuckenberg 1958: 137-143. Zwick 1977: 7-10, 27 (review of morphology and phylogenetics). Arens 1995: 2319-2322, 2326. Arens 1998: 83-84, 90-91, 106, 108-112.

Diagnosis.— Medium-sized blephariceridid. Larva: Cranium dark with L-shaped pale pattern laterally, medial abdominal segments with two rows of minute dorsal tubercles across anterior and posterior borders, intercalary segments with single row of similar tubercles. Pupa: Respiratory organ with 8 distinct lamellae and one transverse bulge; papillae round with corrugated margins. Adult: Wing spur long. Tarsi almost entirely white. Parameres absent; apodeme paddle-shaped, blunt anteriorly.

Description.—Larva (Figs. 2-5, 47, 44-46, 48): Measurements, instar II (n = 1): Total length 1.93mm, cranial width 0.16mm, antennal segment 0.2mm; instar III (n = 16): Total length 3.0mm (1.9-3.8), cranial width 0.26mm (0.25-0.27), basal antennal segment 0.15mm (0.14-0.17), apical antennal segment 0.14mm (0.11-0.17); instar IV (n = 35): Total length 4.7mm (3.1-6.2), cranial width 0.44mm (0.41-046), basal antennal segment 0.25mm (0.22-0.30), apical antennal segment 0.18mm (0.16-0.20). Larva stout with two rows of dark diminutive acutiform dorsal tubercles arranged dorsomedially across abdominal segments 2-6. Cranial sclerites dark brown with a pale L-shaped pattern placed on each side, each reflected; ecdysial lines with little to no stem; posterior margin of frontoclypeal apodeme extended nearly to posterior cranial margin. Remaining cephalothorax uniformly light brown. Apical antennal segment ½ length of basal segment in 4th stage larvae; segments equal in 3rd stage larvae. Abdominal segments and prolegs uniformly light brown; anal division tri-lobed and light brown. Chaetotaxy: posterolateral ends of medial segments with cluster of 6-8 short, stout acutiform sensilla; medial segments with minute acutiform sensilla arranged into

two rows dorsomedially; intercalary segments with one row of acutiform sensilla arranged dorsomedially; anal division lacking sensilla; prolegs with dense apical patch of long, yellow setae.

Pupa (Figs. 25,49-52): Measurement, male (n = 3): Total length 4.0mm (3.9-4.2), total width 2.1mm (1.9-2.2); female (n = 6): Total length 4.9mm (4.3-5.3), total width 2.4mm (2.1-2.7); undetermined (n = 3): Total length 4.2mm (4.0-44), total width 2.1mm (2.0-2.1). Cuticle dark brown; body shape fusiform. Integument: Dorsal papillae uniformly distributed on anal tergite, abdominal segments, metatergite, and alar sclerite; papillae ovoid with corrugated margin, a series of spinelets along posterior margin, smooth dorsally; cuticle between papillae roughly contoured. Respiratory organs elongated transversely; lamellae dark, arranged in a transverse row; 1st lamella apparently modified into a long transverse bulge, followed by 8 distinct separate lamellae, arranged longitudinally; 1st lamella longest, tapering in length until the last, innermost lamellae.

Adult male (Figs. 136, 139-140): Size: small. Measurements (n = 6): Total length 4.2mm (3.9 – 5.0), wing length 6.53mm (6.2 – 7.0), width 2.19mm (2.0 – 2.4).

Leg-Segment Lengths:		foreleg	midleg	hindleg
femur		5.0 (4.8-5.2)	6.2 (6.1-6.5)	7.1 (6.9-7.4)
tibia		4.9 (4.6-5.2)	4.5 (4.2-4.7)	7.2 (7.0-7.5)
tarsus	1	3.2 (3.1-3.4)	2.9 (2.8-3.1)	2.0 (1.9-2.0)
	2	1.0 (0.9-1.0)	1.0 (0.8-1.0)	0.6 (0.6-07)
	3	0.4 (0.4-0.5)	0.5 (0.4-0.5)	0.4
	4	0.2	0.2 (0.1-0.2)	0.2
	5	0.3	0.3 (0.2-0.3)	0.3

Head: Structure: Eyes dichoptic, interoccular distance = 0.241mm (0.171 – 0.259); eyes not divided, ommatidia subequal. Clypeus length/width = 2.1. Proboscis about 0.42x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 0.8 - 1.8 - 2.2. Antennal segments cylindrical; apical segment 1.4x longer than pentultimate segment; scape and pedicel brown with prominent setiforms, remaining flagellomeres dark brown and setose. Chaetotaxy: Post-genals numerous, clypeals numerous.

Thorax (Fig. 136): Wing spur long, 3x length of r-m; Cu and M_4 fused for short distance, anal vein meets wing margin. Fore and midleg with apical portion of first tarsal segment and second, third and 4th tarsal segments pale white in color, hindleg with ¾ of first tarsal segment and second, third and 4th tarsal segments pale white in color; fifth tarsal segment dark brown. Leg segment proportions: foreleg—33:34:21:7:3:1:2, midleg—39:29:19:6:3:1:2, hindleg—39:42:11:3:2:1:2. Chaetotaxy: Thorax glabrous except for dense clumps of setae on the lateral ends of scutellum.

Coloration: Frons and clypeus pale brown; occiput dark; scutum and scutellum lighter brown medially, dark brown along lateral line.

Terminalia (Fig. 139-140): Gonostyle simple, curved sharply towards midline, with abundant setiform sensilla; gonocoxite broad, with abundant setiform; gonocoxal lobes slender, apical portion slightly deflexed; three similar aedeagal rods extend dorsally, slight curve at apex, each covered with a close-fitting tube; vesica ovular; ventral parameres absent; dorsal paramere extends dorsally, t-shaped in cross section; apodeme paddle-shaped, blunt anteriorly; chitinous pillar prominent.

Adult female (Fig. 141): Size: small. Measurements (n = 3) total length 4.75mm (4.6–5.0), wing length 7.4mm (7.3–7.4), width 2.35mm (2.3–2.4).

Leg-Segment Lengths:		foreleg	midleg	hindleg
femur		5.0 (4.8-5.2)	6.2 (6.1-6.5)	7.1 (6.9-7.4)
tibia		4.9 (4.6-5.2)	4.5 (4.2-4.7)	7.2 (7.0-7.5)
tarsus	1	3.2 (3.1-3.5)	2.9 (2.8-3.1)	2.0 (2.9-2.0)
	2	1.0 (0.9-1.0)	1.0 (0.8-1.0)	0.6
	3	0.4 (0.4-0.5)	0.5 (0.4-0.5)	0.4 (0.3-0.4)
	4	0.2 (0.15-0.2)	0.2 (0.1-0.2)	0.2
	5	0.3 (0.28-0.32)	0.3 (0.2-0.3)	0.3

Head: Structure: eyes dichoptic, interoccular distance = 0.30mm (0.25 – 0.33mm); eyes not divided, ommatidia subequal. Clypeus length/width = 3.03. Proboscis about 0.56x head width; mandible finely serrated along inner edge; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - .08 – 2.1 – 2.15. Antennal segments cylindrical; apical segment 1.6 longer then pentultimate segment; scape and pedicel brown with prominent setiforms, remaining flagellomeres dark brown and setose. Chaetotaxy: Post-genals numerous, clypeals numerous.

Thorax: similar to male in appearance and wing venation.

Coloration: Frons pale brown. Clypeus with pale circle at base. occiput dark; scutum and scutellum lighter brown medially, dark brown along lateral line.

Terminalia (Fig. 141): Hypogynial valve broad basally, narrowed apically into two narrowed, valves; individual valves rounded lobes. Accessory gland elongate. Spermathecae

3 in number, subspherical, simple. Chaetotaxy: Sparse setae across sternite VIII; hypogynial plate and valves pruinose.

Type Material.—Holotype [adult male, pinned]: MADAGASCAR: *Toamasina*: "Perinet", December 1955, coll. B. Stuckenberg [IRSM]. Specimens collected from a small stream "few miles east" of "Perinet", now known as the village of Andasibe next to Mandtadia National Park. "Stream flowed into larger river that flowed past the village". "Site 800m from large sawmill." Allotype [adult female, pinned]: same data a holotype [IRSM]. Paratypes: [10 male A (pinned), 4 female A (pinned), 4 male A (dissected), 3 female A (dissected), numerous larvae and pupae]: Same data as holotype. Paratypes deposited in IRSM and NM.

Other material examined.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004, 13 June 2006, 6 January 2007 [LPA]; *Toamasina*: "Perinet", 1955, coll. B. Stuckenberg; Mantadia National Park, S18°49.717' E48°26.417', 16 October 2004 [L], 15 June 2006 [L]; Analamazaotra Special Reserve, S18° 55.717' E48° 25.467', 16 June 2006 [L]; Vohimana Reserve, S18°56.389" E48°30.999', 29 June 2006, Relais du Naturaliste, Iasina River, [L]; Vohimana Reserve, S18°55.273' E48°30.770', 29 June 2006, Relais du Naturaliste, Vohimana R @ stream xing [L]; Vohimana Reserve, S18°55.362' E48°30.72', 29 June 2006, Vohimana R., falls next to village [L]; Vohimana Reserve, S18°55.462' E48°30.58', 29 June 2006, Tsat sahina flowing into Vohimana R. [LP]; Vohimana Reserve, 03 Jan 2007, Village de Chercher/Nursery [LPA]; Sahatandra R., S18°54.384' E48°28.83', trib. into river [L]; *Fianarantsoa*: Ranomafana National Park, S21°15.86' E47°24.69', 10-11 October 2004 Sacre Roa and trib. [L]; Ranomafana, S21°15.232' E47°27.447', 12 October 2004, village waterworks [L].

Specimens from “Perinet” (now known as Andasibe), 1955 were collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Paulianina umbra* is widely distributed across Madagascar, though it has not been collected in northern regions. It is much more abundant than either *P. hova* or *P. ingens*. It is sympatric with *P. ingens* and many *Eupaulianina* species, and can be collected from the same rock with several other species. When collected among other species, *P. umbra* is usually present in higher numbers than *P. ingens*, but is much less abundant than *Eupaulianina* species.

Bionomics.—Larvae can be collected during all seasons, with adults collected in the highlands as early as October, and in the eastern forests during the rainy season (December-January). The difference in temperatures between the highland site and the eastern forest could influence adult emergence, and would explain the early emergence at the highland site (ASR). Larvae and pupae are usually collected in the fast flowing water, cascades, and waterfall faces of smaller streams that are heavily shaded. Larvae were also collected from a small cave in MNP, where thin films of water ran down the cave walls. Pupae often clustered together, facing upstream, along the edge of water flow. Adults were often easy to see in the shade because to their white tarsi, which reflect light. Many flies hung by the tarsi of the their front legs from spider webs on the undersides of rocks and trees over the streams allowing hind legs hanging down, with subsequent flies attaching to those hind legs with their front tarsi, creating long chains of adults. When disturbed, flies start to vibrate, causing the chain to bob up and down. Adults are active mainly in morning hours, and display a flight pattern that is characteristic of the family- a dancing flight in mid-air over the foaming cascades and over stones in mid-stream.

***Paulianina lehibe* sp. nov.** Sam and Courtney (Figs. 6, 26, 53-60)

Diagnosis.— Very large blephariceridid. Larva: Dorsal surface of medial segments with four dark prominent sharp sensilla distributed evenly in horizontal line; intercalary segments with dark, tall, sharp sensilla placed medially in line with the two center sensilla on the medial segments. Pupa: Respiratory organ with nine separate plate-like lamellae, spaced evenly.

Description.—Larva (Fig. 6): Measurements, instar II (n = 1): Total length 2.8mm, cranial width 0.33mm, basal antennal segment 0.23mm, apical antennal segment 0.23mm; instar III (N=5): Total length 4.6mm (3.7-5.8), cranial width 0.36mm (0.35-0.39), basal antennal segment 0.23mm (0.20-0.25), apical antennal segment 0.25mm (0.23-0.3); instar IV (N=5): Total length 9.8mm (7.7-13.0), cranial width 0.70mm (0.48-0.80), basal antennal segment 0.7 (0.6-0.76), apical antennal segment 0.3mm (0.28-0.31). Cranial sclerites uniformly light brown with black border along posterior margin; ecdysial lines with little to no stem; posterior margin of frontoclypeal apodeme extended nearly to posterior cranial margin; remaining cephalothorax pale. Abdominal segments and prolegs uniformly pale; anal division pale. Anal division tri-lobed, not divided; outer lobes blunt; center lobe an acute triangle with lobed apex. Chaetotaxy: dorsal surface of medial segments with four dark prominent sharp sensilla distributed evenly in horizontal line; intercalary segments with dark, tall, sharp sensilla placed medially in line with two center sensilla on medial segments; prolegs with dense apical patch of long, yellow setae.

Pupa (Figs. 26, 53-60): Measurements, male (n = 1): Total length 6.5mm, width 3.1mm; undetermined (n = 1): Total length 7.1mm, total width 3.3mm. Cuticle dark brown; body

shape fusiform. Integument: dorsal papillae uniformly distributed on anal tergite, abdominal segments, metatergite, and branchial sclerite; papillae with multiple ridges, some with a curved sharp dorsal spine; cuticle between papillae roughly contoured. Respiratory organs elongated horizontally, with 9 lamellae; lamellae, dark, arranged transversely; lamella 1 broad, rounded, interior margin projected medially; lamellae 2-9 plate-like, ovoid, spaced evenly.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Fianarantsoa*: Antanifotsy, Andringitra National Park, Kimoro R on east slopes of Ambaravarandanitra Mt., 19 January 1958, coll. B. Stuckenberg. **Other material examined**.—MADAGASCAR: *Fianarantsoa*: Antanifotsy, Andringitra National Park, Kimoro R on east slopes of Ambaravarandanitra Mt., 19 January 1958 [L]; Antanifotsy, Andringitra National Park, Jomando R., 10 January 1958 [L]. All specimens collected by B. Stuckenberg.

Distribution.—*Paulianina lehibe* seems to be limited to high altitudes in ANP. No specimens were collected in the same location during recent expeditions, so it is possibly extinct. Former records indicate possible sympatry with *P. ingens*, *E. alexanderi*, *E. pamela*, *E. silva*, *E. botsimpatsy*, *E. korontsilo*, and *E. telofantsy*.

Bionomics.—Larvae were collected in January from large streams in ANP.

Etymology.—From Malagasy for “large” (lehibe), in reference to the fact that it is the largest larval species known from Madagascar.

***Paulianina ratsipika* sp. nov.** Sam and Courtney (Fig.7)

Diagnosis.—Large blephariceridae. Larva: Ecdysial lines with no stem; posterior margin of frontoclypeal apodeme, blunt, extended to posterior cranial margin. Both medial and intercalary segments with central sensilla clustered so closely together to appear as a long thin plate.

Description.— Larva (Fig. 7): Measurements, instar III (n = 1): Total length 2.9mm, cranial width 0.28mm, basal antennal segment 0.05mm, apical antennal segment 0.1mm; instar IV (n = 2): Total length 4.3mm, cranial width 0.42mm, basal antennal segment 0.17mm, apical antennal segment 0.12mm. Cranial sclerites uniformly light brown with black border along posterior margin, ending well before ecdysial lines; ecdysial lines with no stem; posterior margin of frontoclypeal apodeme, blunt, extended to posterior cranial margin. Remaining cephalothorax quadrate, large and pale. Abdominal segments and prolegs uniformly pale; anal division tri-lobed, pale, not divided; outer lobes blunt, center lobe broadly rounded. Chaetotaxy: Cephalothorax with three horizontal rows of minute, stout, pointed, dark sensilla; dorsal surface of medial segments with two horizontal rows of short, stout, pointed, dark sensilla; anterior row with central sensilla clustered so closely together to appear as a long thin plate; posterior row of sensilla separate. Intercalary segment with central single horizontal line of short, stout, pointed, dark sensilla clustered so closely together to appear as a long thin plate, covering middle half of segment; lateral ends of intercalary segments with scattered short, stout, pointed, dark sensilla. Dorsal surface of prolegs with scattered short, stout, pointed, dark sensilla; prolegs with dense apical patch of long, yellow setae.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Toamasina*: dist. Maroantsetra, Ambodivohangy, 16-20 March 1958, coll. B. Stuckenberg. **Other material examined**.—*Toamasina*: dist. Maroantsetra, Nosy Mangabe, 30 March 1958, [L] coll. B. Stuckenberg.

Distribution.—*Paulianina ratsipika* seems to be restricted to the region surrounding Maroantsetra, at the northern end of Helodrano Antonglia Bay. Ambodivohangy is approximately 60 km north-west of Maroantsetra. Nosy Mangabe is a small island approximately 4km south of Maroantsetra. The species is sympatric with *P.* species J, *E. rivalis*, *E. silva*, *E. pamela*, *E. botsimpatsy*, *E. borivody, E. telofantsy.*

Bionomics.—Larvae were collected in January.

Etymology.—From Malagasy for "line" (tsipika), in reference to the appearance of horizontal lines across the dorsum of the larva.

***Paulianina* species J** (Fig. 8)

Diagnosis.— Large blephariceridae. Larvae dorsally glabrous, without prominent chateotaxy; central portion of anal division triangular.

Description.—Larva (Fig. 8): Measurements, instar IV (n = 4): Total length 6.6mm (4.3-8.8), cranial width 0.58mm (0.56-0.6), basal antennal segment 0.15mm (0.12-0.16), apical antennal segment 0.13mm (0.08-0.16); Larva stout. Cranial sclerites uniformly dark brown with black border along posterior margin; ecdysial line with little to no stem; posterior margin of frontoclypeal apodeme extended nearly to posterior cranial margin. Remaining

cephalothorax uniformly pale. Abdominal segments and prolegs uniformly pale; anal division pale, tri-lobed, not divided; outer lobes blunt; center lobe triangular. Chaetotaxy: Dorsal surface of medial and intercalary segments covered with random arrangement of sharp minute sensilla; prolegs with dense apical patch of long, yellow setae.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Antananarivo*: Antsirabe, Col de Tapia, 29 February 1958, coll. B. Stuckenberg. **Other material examined**.—MADAGASCAR: *Antananarivo*: Antsirabe, Col de Tapia, 29 February 1958 [L], coll. B. Stuckenberg.

Distribution.—Species J seems to be limited to central Madagascar. No specimens were collected in the same location during recent expeditions, so it is possibly extinct. Former records suggest sympatry with *P. ratsipika*, *E. silva*, and *E. telofantsy*.

Bionomics.—Adults were collected in January in central Madagascar in fairly large streams.

Species Descriptions: *Eupaulianina* (Stuckenberg 1958)

Eupaulianina vohimalama Sam and Courtney, new species (Figs. 21, 113-115)

Diagnosis.—Larva: Pale in coloration, small smooth plated present dorsally with only minute tubercle sensillum at lateral ends. Anal division tri-lobed, similar to *Paulianina*,

without distinct prolegs. Pupa: Narrowed at alar sclerite; truncated anal sclerite; respiratory organ similar to *Paulianina ingens* in orientation and structure, but with only six lamellae.

Description.— Larva (Figs. 21, 113): Measurements, instar IV (n = 5) total length 55mm (47-63), cranial width 0.52mm (0.49-0.54), first antennal segment 0.15mm (0.13-0.16), second antennal segment 0.13mm (0.11-0.14). Larva slender. Cranial sclerites light brown; posterior margin with dark brown border, produced anteriorly 1/3 length from midline; ecdysial line with very short stem; posterior margin of frontoclypeal apodeme does not reach posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed at midline. Coloration: Body uniformly pale. Raised plates light brown, similar in color to cranial sclerites. Lateral lobes with prolegs visible dorsally. Chaetotaxy: No spines or tubercles present; AD absent; AL present as small, dark round impression; LP present as smooth, light brown, circular plate; M present as subrectangular, smooth, light brown plate; AM smooth ovoid plate with minute tubercle sensillum at lateral margins; PM present as subrectangular, smooth, light brown plate, narrower and 0.6x length of AM; PP present only has small circular impression, slightly darker in color then surrounding integument; R present as two subrectagular plates, similar in size and shape to AM and PM, respectively; S present as two subequal subrectangular smooth, light brown plate; T present as dark brown posterior border. Clear division between last abdominal segment and anal segment; anal division tri-lobed, without distinct prolegs; outer lobes pointed with dark brown apex. Prolegs on cephalothorax tapered; abdominal prolegs present, stout, conical dark brown, with dense apical patch of long, yellow setae.

Pupa (Figs. 114, 115): Measurements, undetermined (n = 1): Total length 4.4mm, width 2.1mm. Cuticle dark brown. Body roughly ovoid, narrowing at alar sclerites; Third

abdominal segment swollen at lateral ends. Anal segment truncated along posterior margin. Papillae present, uniformly dispersed across cephalic sclerite, abdominal tergites, and anal tergite; absent on alar sclerite. Respiratory lamellae dark brown. Respiratory organs elongated transversely. Respiratory organ with six distinct swollen lamellae. Lamellae run longitudinally, lamellae one and six curve posteriolaterally towards each other, meeting posteriolateraly, forming a basket-like structure. Lamellae two, three, four and five parallel to each other, curving posteriolaterally margin of respiratory organ; lamellae two and five 0.8x length of outer lamellae; lamellae three ½ length of outer lamellae; lamellae four 0.6x length of outer lamellae.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Toamasina*: Vohimana Reserve, S18°56.389' E48°30.999', 29 June 2006, Relais du Naturaliste, Iasina River, coll. R. Sam. **Other Material examined**.—MADAGASCAR: *Toamasina*: Vohimana Reserve, S18°56.389' E48°30.999', 29 June 2006, Relais du Naturaliste, Iasina River, [L]; *Fianarantsoa*: Ambalavao, 1958, Jomando R. Specimens collected from Ambalavao collected by B. Stuckenberg; specimens from Vohimana collected by R. Sam.

Distribution.—*Eupaulianina vohimalama* has a limited distribution, only being collected in two locations (VSR and Ambalavao), though the locations are widely geographically separated.

Bionomics.—*Eupaulianina vohimalama* larvae collected in a thin flow of water flowing over a large bedrock sheet at VSR, data unknown for Ambalavao. It is sympatric with *P. ingens*, *P. umbra*, *E. alexanderi*, *E. tsilobe*, and *E. marobotsin*.

Etymology.—From Malagasy for “smooth hill” (vohimalama), in reference to the smooth dorsal plates that are unique to this species.

Eupaulianina silva group (Stuckenberg) 1958

Included species.—*Eupaulianina*:

alexanderi (Stuckenberg) (tentatively placed based on adult genitalic structures)

silva (Stuckenberg)

tsilobe new species

telofantsy new species

fantsona new species

Diagnosis.—Larva: Chaetotaxy consists of three spines places on the AM plate, spnes elongate tipped with pointed sensilla; Pupa: Papillae with either globose or lanceolate projections from the base of spinelets; respiratory organs kidney shaped with 5-6 lamellae present as swellings with moderate to little space between. Adult: Lateral aedeagal filaments enter parameres, medial filament encased in independent tube.

Eupaulianina alexanderi (Stuckenberg) comb. nov. (Figs. 11, 28, 75-82, 143, 144)

Syn: *Paulianina alexanderi* Stuckenberg 1958

Eupaulianina alexanderi (Stuckenberg) 1958: 172-177. Zwick 1977:10 (review of morphology and phylogenetics). Arens 1998: 84, 101-105, 107, 109-112.

Diagnosis.—Large blepharicerid. Larva: Chaetotaxy consists of dark stout conical sensillum, anal division with 10-15 smaller tubercles. Pupa: Respiratory organ simple, lamellae mostly merged, papillae well developed, round, with single lanceolate tubular

processes directed posteriorly from a base of spinelets. Adult: Wing spur approximately the length of r-m; aedeagal filaments enter ventral parameres; medial aedeagal filament absent.

Description.— Larva (Figs. 11, 75-78): Measurements, instar II (n = 1): Total length 2.5mm, cranial width 0.23mm, antennal segment 0.16mm; instar III (n = 23): Total length 2.9mm (2.2-3.8), cranial width 0.36mm (0.31-0.46), antennal segment 0.29mm (0.23-0.38); instar IV (n = 54): Total length 5.1mm (3.2-6.0), cranial width 0.54mm (0.46-0.59), basal antennal segment 0.14mm (0.12-0.15), apical antennal segments 0.25mm (0.23-0.31). Cranial sclerites dark brown with black margin posteriorly; ecdysial line with short stem line; frontoclypeal apotome does not extend to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed at midline. Coloration: Variable; uniformly light brown medially, with lateral lobes becoming pale yellow brown apically. Lateral lobes with prolegs visible dorsally. Chaetotaxy: cranial sclerite with group of small conical tubercles arranged posteriolaterally; AD consists of two independent darkly colored conical tubercles place on a dark ovoid plate-like sclerite; AL with 6-9 conical tubercles arranged in order of descending size in marginal direction, each tipped with a setiform sensillum; M composed of a plate-like sclerite with two conical tubercles, each tipped with a setiform sensillum, 1-2 smaller conical tubercles radiating laterally from sclerite; LP base pale yellow in coloration with 3 dark conical tubercles radiating outwards, additional tubular processes may be present on anterior face of base; AM consists of large, dark plate-like sclerite with three large conical tubercles, largest tubercles in middle, each tipped with a setiform sensillum, additional tubular processes present at the base of each tubercle; PM with two conical tubercles placed laterally on narrow dark plate-like sclerite, tubercles smaller then those on AM sclerite; PP consists of one dark conical tubercle tipped

with a single setiform sensillum; Conical tubercles of R, arranged three anteriorly and two posteriorly, situated on dark, reverse trapezoid-shaped plate-like sclerite, middle anterior tubercle largest, all tubercles tipped with a setiform sensillum; T composed of two conical tubercles, each tipped with a setiform sensillum, placed laterally on dark square plate-like sclerite; all plate-like sclerites dark in color with shagreened surface; intercalary segments with 2-3 small conical tubercles placed apically; lateral lobes with numerous small tubercles bearing stiff setiform sensillum.

Pupa (Figs. 28, 79-82): Measurements, male (n = 10): Total length 3.82mm (3.6-4.2), width 2.0 (1.9-2.1); female (n = 4): Total length 4.02mm (3.9-4.2), width 2.0mm (1.9-2.2); undetermined (N=22): Total length 4.0mm (3.5-4.5), width 2.2mm (2.1-2.3). Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Integument: Dorsal papillae present, uniformly dispersed across all branchial sclerite, cephalic sclerite, abdominal tergites, and anal tergite; absent on alar sclerite. Papillae well developed, glabrous; ovoid, flattened on top; single lanceolate projection extended posteriorly from base of spinelets. Cuticle between papillae homogenous without particular patterning, uniform in pigmentation. Respiratory lamellae dark brown, simple structure; lamellae merged into kidney-shape, with flattened edge along ½ posterior margin; lamellae present as 3 swellings closely placed, with very narrow divisions between; two small, Y-shaped divisions to indicate respiratory suture, beginning at the intersection of the flattened and ovular edge.

Adult male (Fig. 143): Size: medium. Measurements (n = 1): Total length 3.4 mm, wing length 6.7 mm, width 2.2 mm.

Leg –

Segment

Lengths:	foreleg	midleg	hindleg
femur	4.0	5.0	5.8
tibia	4.5	4.1	6.0
tarsus 1	2.0	2.1	1.3
2	---	0.7	0.4
3	---	0.3	0.2
4	---	0.2	0.1
5	---	0.2	0.2

Head: Structure: Eyes dichoptic, interoccular distance = 0.21 mm; eyes not divided, ommatidia subequal. Clypeus length/width = 2.4. Proboscis about 0.1x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 0.9 - 1.5 - 1.9. Antennal segments cylindrical; apical segment 1.1x longer then pentultimate segment; scape and pedicel both brown with prominent setiforms, remaining flagellomeres dark brown and setose. Chaetotaxy: Post-genals (numerous), clypeals (numerous).

Thorax: Wing spur short, approximately length of r-m; M_3 extends 0.6 length inwards; anal vein does not reach wing margin, may be extended by microtrichia. M_4 and Cu_1 fuse for a very short distance; last tarsomere of middle leg subequal to penultimate segment.

Coloration: Frons and clypeus pale brown; occiput dark; scutum and scutellum lighter dark brown.

Terminalia (Fig. 143): Gonostyles curved broadly towards midline, with abundant setiform sensilla; gonocoxite broad, with abundant setiform sensilla along lateral boundary; gonocoxal lobes elongate, rounded at apex, flexed posteriorly in straight line; ventral bridge present as broad semi-circlular shape with lateral arm broad at base, narrowing apically;

dorsal paramere subquadrate with shallow depression where parameres lie; vesica elongate; aedeagal filaments lanceolate, lateral filaments projected at angles from central filament; ventral parameres curved in loose s-shape; lateral aedeagal filaments enter ventral parameres, medial filament absent; medial tube present, but empty; apodeme reduced, with ridge down median line.

Adult female (Figs. 144): Size: Medium. Measurements (n = 4): Total length 4.6 mm (4.6-4.7), wing length 7.0 mm (6.9-7.1), width 2.3 mm (2.3-2.4).

Leg –

Segment

Lengths:		foreleg	midleg	hindleg
femur		4.6 (4.5-4.9)	5.7 (5.3-6.0)	6.4 (6.3-6.5)
tibia		4.9 (4.7-5.0)	4.1 (4.0-4.4)	6.8 (6.6-7.0)
tarsus	1	2.3 (2.1-2.3)	2.0 (2.0-2.1)	1.3 (1.3-1.4)
	2	0.7 (0.6-0.7)	0.7 (0.6-0.7)	0.5 (0.4-0.5)
	3	0.4 (0.3-0.4)	0.3 (0.2-0.3)	0.24
	4	0.19 (0.16-0.20)	0.20	0.14 (0.12-0.16)
	5	0.2 (0.2-0.3)	0. 22 (0.22-0.24)	0.22 (0.20-0.24)

Head: Structure: Eyes dichoptic, interoccular distance = 0.28 mm; eyes not divided, ommatidia subequal. Clypeus length/width = 2.9. Proboscis about 0.52x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 0.8 - 1.4 - 2.4. Antennal segments cylindrical; apical segment 1.2x longer then pentultimate segment; scape and pedicel both brown with prominent setiforms, remaining flagellomeres dark brown and setose. Chaetotaxy: Post-genals (numerous), clypeals (numerous).

Thorax: Same as in male, except for M_4 and Cu_1 fuse for a even shorter distance. Last tarsomere of middle leg 1.75x penultimate segment; stout basal spines spread over basal 1/3 of segment.

Terminalia (Fig. 144): Eighth sternite with elongate window at the base of the median fold. Hypogynial valve broad basally, narrowed apically into two valves; individual valves broad. Accessory gland elongate. Spermathecae 2 in number,spherical, with wide tubular neck whose walls are roughened and thickened by many fine rings.. Chaetotaxy: Sparse setae across sternite VIII; hypogynial plate and valves pruinose.

Type Material.—Holotype [adult male, pinned]: MADAGASCAR: *Antananarivo*: Manjakatompo Forest Station, Ankaratra massif, Antsomangana R. 4-10 January, 1956, coll. B. Stuckenberg. Paratypes [1 pupa (dissected), numerous larvae and pupae]: data same as holotype.

Other material examined.—MADAGASCAR: *Toamasina*: Mantadia National Park, S18°49.717' E48°26.417', 16 October 2004 [L], 15 June 2006 [L]; Analamazaotra Special Reserve, S18° 55.717' E48° 25.467', 16 June 2006 [L]; Vohimana Reserve, S18°56.389" E48°30.999, 29 June 2006, Relais du Naturaliste, Iasina River, [L]; Vohimana Reserve, S18°55.273' E48°30.77', 29 June 2006, Relais du Naturaliste, Vohimana R @ xing [L]; Vohimana Reserve, S18°55.362' E48°30.72', 29 June 2006, Vohimana R., falls nxt to village [L]; Vohimana Reserve, S18°55.462' E48°30.586', 29 June 2006, Tsat sahina flowing into Vohimana R. [LP]; Vohimana Reserve, 03 Jan 2007, Village de Chercher/Nursery [LPA]; Sahatandra R., 04 Jan 2007, S18°54.384' E48°28.83', trib. into river [L]; Ivontaka, 1958, Antanambe; *Fianarantsoa*: Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January 2007, Riambavy, Riandahy, Zomandao [LPA]; Ambalavao, 1958, Jomanda R.;

Ambalavao, 1958, Vakona R.; Ambalavao, 1958, Ambaravarandanitra Mt.; Ambalavao, 1958, nr Antanifotsy. Specimens from Majakatompo, Ivontaka and Ambalavao collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Eupaulianina alexanderi* is the most commonly collected Malagasy blephariceridae. It is widely distributed across central, eastern and southeastern regions. It is found in both low and high elevation streams. It occurs in both small and large streams, and has even been collected on a small cave wall moistened by trickling water (MNP). The species is sympatric with *Paulianina* s. str., though they greatly outnumber *Paulianina* s. s. when collected on the same rock. It is sympatric with *P. umbra*, *P. ingens*, *E. rivalis*, *E. pamela*, *E. silva*, *E. tsilobe*, *E. marobotsin*, *E. tandroka*, *E. telofantsy*, and *E. botsimpatsy*.

Bionomics.—*Eupaulianina alexanderi* is fairly active all year, with the peak adult activity occurring in the morning hours during December-February. Larvae and pupae originally described by Stuckenberg as collected in larger streams, on more submerged rocks, similar to *P. ingens*. However, this did not seem to be the strict case in the 2004-2007 collecting trips, with larvae being collected in a wide variety of habitats, stream sizes, and elevations, generally found on rock surfaces covered with a fairly thin flow of water. Stream conditions range from small, shaded streams to larger streams. Adults active mainly in morning hours, and display flight pattern that is characteristic of the family, a dancing flight in mid-air over the foaming cascades and over stones in mid-stream.

Eupaulianina silva (Stuckenberg) comb. nov. (Figs. 14, 29, 91, 93, 95, 97, 146)

Syn: *Paulianina silva* Stuckenberg 1958

Eupaulianina silva Stuckenberg 1958: 167-172. Zwick 1977: 10 (review of morphology and phylogenetics). Arens 1998: 84, 99-101, 107, 109-112.

Diagnosis.—Medium blephariceridid. Larva: AM composed of two large spines set on lateral ends of large ovoid plate and a single smaller spine medially, inner spine ¼ length of outer spines, outer spines curved and proclinate. Pupa: Papillae with single globose projection extended posteriorly on a slender stem from spinelets; respiratory organ kidney-shaped; lamellae present as 6 swellings with narrow space between. Adult: Wing spur short; ventral bridge appears ovoid ventrally, with two invaginations posteriorly; ventral plate appears as half circle with lobes at lateral apices; lateral aedeagal filaments enter ventral parameres, medial aedeagal filament encased in slender tube, bulbous at base.

Description.—Larva (Figs. 14, 91): Measurements, instar II (n = 1): Total length 1.82mm, cranial width 0.40mm, antennal segment 0.10mm; instar III (n = 1): Total length 2.4mm , cranial width 0.27mm, antennal segment 0.22mm; instar IV (n = 5): Total length 5.0mm (3.0-6.0), cranial width 0.63mm (0.6-0.7), basal antennal segment 0.07 (0.6-0.8), apical antennal segment 0.16mm (0.15-0.17). Larva slender with elongate spines across body. Cranial sclerites dark brown; ecdysial line with short stem; posterior margin of frontoclypeal apodeme does not reach posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed at midline. Coloration: Body uniformly light brown. Raised plates brown; spines piceous. Lateral lobes with prolegs visible dorsally. Chaetotaxy: Larval integument strigate on abdominal segments, glabrous on intercalaries; body with a series of elongate spines on raised, glabrous plates, spines with long, glabrous base, tipped with pointed, elongate striated sensillum; AL composed of two conical tubercles set in horizontal line; AD composed of two large spines set on elongate

oval plate, inner spine twice as long as outer spine, both spines curved and proclinate; M composed of two single spines, each set on lateral ends of narrow ovoid plate; LP composed of single long spine, curved inwardly; AM composed of two large spines set on lateral ends of large ovoid plate and a single smaller spine medially, inner spine ¼ length of outer spines, outer spines curved and proclinate; PM composed of two small spines on narrow ovoid plate narrow medially, spines similar in size to inner AM spine; PP a single spine, ½ length of PM spines; R subquadrate with transverse furrow across middle, a single erect spine set in each corner ; S similar to M, composed of two single spines, each set on lateral ends of narrow ovoid plate; T composed of two erect, small spines; intercalary segments with 2 small spines on lateral corners; prolegs with several small spines scattered dorsally and dorsolaterally, tipped with dense apical patch of long, yellow setae.

Pupa (Figs. 29, 93, 95, 97): Measurements, male (n = 1): Total length 3.88mm, width 2.13mm; female (n = 5): Total length 4.46mm (4.32-4.57); undetermined (n = 4): Total length 4.0mm (3.75-4.38), width 2.1mm (1.88-2.25). Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Integument: Dorsal papillae present, uniformly dispersed across all branchial sclerite, cephalic sclerite, abdominal tergites, and anal tergite; absent on alar sclerite. Papillae well developed, glabrous; ovoid, with small clump of spinelets present along posterior margin; single globose projection extended posteriorly on a slender stem from spinelets. Cuticle between papillae homogenous, without particular patterning; uniform in pigmentation. Respiratory organ dark brown, kidney-shaped; lamellae present as 6 swellings with narrow space between; 1st lamellae largest, 3x width of other lamellae 2-6; 6th lamellae extends around base of lamellae 3-5, with apex at the juncture of lamellae 2 and

3; respiratory suture begins at the basal juncture of lamellae 2 and 3, continuing along the base of lamellae 4 and 3, to end at between lamellae 3 and 4.

Adult male (Fig. 146): Size: Medium. Measurements (n = 1): Total length 3.0 mm, wing length 4.3mm, width 1.5mm.

Leg –

Segment Lengths:		foreleg	midleg	hindleg
femur		4.4	5.1	6.0
tibia		4.3	3.9	6.2
tarsus	1	2.2	2.1	—
	2	0.68	0.72	—
	3	0.32	0.32	—
	4	0.20	0.16	—
	5	0.20	0.20	—

Head. Structure: Eyes dichoptic, interoccular distance = 0.28 mm; eyes not divided, ommatidia subequal. Clypeus length/width = 2.8. Proboscis about 0.3x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 0.8 - 1.3 – 1.9. Antennal segments cylindrical, scape and pedicel both brown with prominent setiforms, remaining flagellomeres dark brown and setose. Chaetotaxy: post-genals (numerous), clypeals (numerous).

Thorax. Wing spur short, 0.5x length of r-m; M_3 extends 0.6 length inwards; Cu_1 and M_4 fused for short distance, anal vein meets wing margin. Last middle tarsomere 1.5X penultimate segment.

Coloration. Frons and clypeus pale brown; occiput dark; scutum and scutellum lighter brown medially, dark brown along lateral line.

Terminalia. Gonostyles curved sharply towards midline, with abundant setiform sensilla; gonocoxite long, with abundant setiform sensilla along lateral boundary; gonocoxal lobes elongate, rounded at apex, flexed inward; ventral bridge appears ovoid ventrally, with two invaginations posteriorly; ventral plate appears as half circle with lobes at lateral apices; dorsal paramere elongate with distinct rounded lobes at lateral apices, slight rounding along midline; vesica hourglass-shaped; ventral parameres rounded distally at base, curving inward, slightly lobed at apex; lateral aedeagal filaments enter ventral parameres, medial aedeagal filament encased in slender tube, bulbous at base; apodeme lobed, with ridge down median line.

Adult female: (Based entirely on Stuckenberg 1958) (Fig.): Size: medium. Wing length = 6.75mm; Wing width = 2.62mm.

Terminalia. Eighth sternite with without elongate window at the base of the median fold. Hypogynial valve elongate, apical lobes narrow. Spermathecae 3 in number, with annular, cup-shaped head and wide tubular neck whose walls are roughened and darkened by many fine ridges.

Type Material.—Holotype [adult male, pinned]: MADAGASCAR: *Antananarivo*: Manjakatompo Forest Station, Ankaratra massif, Antsomangana R. 4-10 January, 1956. Allotype [adult male pinned]: same data as holotype. Paratypes [1 male A (pinned), 1 male A (dissected), numerous larvae and pupae]: data same as holotype. Paratypes deposited in IRSM and NM.

Other material examined.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LPA], 13 June 2006 [L], 6 January 2007 [LPA]; Antsirabe, 1958, Col de Tapia; *Fianarantsoa*: Andringitra National

Park, S22° 08.73' E46° 53.46', 10-11 January 2007, Riambavy, Riandahy, Zomandao [LPA]; Ranomafana National Park, S21°15.86' E47° 24.69', 10-11 October 2004 Sacre Roa and trib. [L]; Ranomafana, S21°15.232' E47°27.447', 12 October 2004, village waterworks [L]; Ambalavao, 1958, Jomando R. Specimens from Antsirabe and Ambalavao collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.— *Eupaulianina silva* has a locally limited distribution yet is collected in three separate provinces. It is collected at AMSP, ANP, RNP and areas between. *E. silva* is sympatric with many species, including *P. umbra*, *E. rivalis*, *E. pamela*, *E. tsilobe*, *E. tandroka*, *E. marobotsin*, and *E. telofantsy*.

Bionomics.—Larvae and pupae originally described by Stuckenberg as collected in larger streams, on more submerged rocks, similar to *P. ingens*. However, this did not seem to be the strict case in the 2004-2007 collecting trips, with larvae being collected in a wide variety of habitats, generally found on rock surfaces covered with a fairly thin flow of water. Stream conditions range from small, shaded streams to larger streams. Adults active mainly in morning hours, and display flight pattern that is characteristic of the family, a dancing flight in mid-air over the foaming cascades and over stones in mid-stream.

Eupaulianina telofantsy Sam and Courtney, new species (Figs. 16, 31, 99-106)

Diagnosis.—Medium blephariceri. Larva: AM with three spine-like tubercles tipped with dark, pointed sensillum of similar size. Pupa: Respiratory organ kidney-shaped with 5 swellings; papillae ovoid, scalloped along margin; small clump of spinelets present along posterior margin; single, elongo-elliptoid projection extended from posterior margin on a slender stem from group of spinelets; integument reticulate.

Description.—Larva (Figs. 16, 99-102): Measurements: instar II (n = 3): Total length 1.75mm (1.53-1.93), cranial width 0.22mm, antennal segment 0.132mm (0.10-0.15); instar III (n = 10): Total length 3.01mm (2.56-3.68), cranial width 0.37 (0.32-0.4), antennal segment 0.18mm (0.15-0.27); instar IV (n = 15): Total length 5.52mm (4.4-6.3), cranial width 0.60mm (0.56-0.64), basal antennal segment 0.10 (0.08-0.11), apical antennal segment 0.16mm (0.14-0.27). Larva slender with elongate spines across body. Cranial sclerites dark brown, posterior margin produced posteriorly in median line into a short lobe, occupy ½ the space between plates AD; ecdysial line with short stem; posterior margin of frontoclypeal apodeme does not reach posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Coloration: Body uniformly light brown. Raised plates brown; spines piceous. Lateral lobes with prolegs visible dorsally. Chaetotaxy: Larval integument strigate on both abdominal segments and intercalaries; body with a series of narrow elongate spines on raised, glabrous plates, spines with long, glabrous base, tipped with pointed, elongate striated sensillum; AL composed of three conical tubercles set in horizontal line; AD composed of two large spines set on elongate oval plate, inner spine twice as long as outer spine, both spines curved and proclinate; M composed of two single spines, each set on lateral ends of narrow ovoid plate; LP composed of single long spine, curved inwardly; AM composed of three large spines set evenly on large ovoid plate, spines curved and proclinate; PM composed of two small spines on narrow ovoid plate narrow medially, spines similar in size to inner AM spine; PP a single spine, ½ length of PM spines; R subquadrate with transverse furrow across middle, a single erect spine set in each corner ; S similar to M, composed of two single spines, each set on lateral ends of narrow ovoid plate; T composed of two erect, small spines; intercalary

segments with 2 small spines on lateral corners; prolegs with several small spines scattered dorsally and dorsolaterally, tipped with dense apical patch of long, yellow setae.

Pupa (Figs. 31, 103-106): Measurements, male (n = 6): Total length 3.78mm (3.6-3.9), width 2.13mm (2.0-2.3); female (n = 15): Total length 4.42mm (4.2-4.7), width 2.33mm (2.3-2.5). Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Integument: Dorsal papillae present, uniformly dispersed across cephalic sclerite, abdominal tergites, and anal tergite; absent in margin around respiratory organ; absent on alar sclerite. Papillae well developed, glabrous; ovoid, scalloped along margin; small clump of spinelets present along posterior margin; single, elongo-elliptoid projection extended from posterior margin on a slender stem from group of spinelets. Papillae on branchial sclerites distinct from other papillae; ovoid with strongly scalloped edges; slender elliptoid projection extended from point between posterior margin and midpoint on papillae. Cuticle between papillae reticulate, uniform in pigmentation. Respiratory lamellae dark brown, kidney-shaped; lamellae present as 5 swellings, adjacent, with no gaps or space; 1st lamellae, large and pointed at apex, at least 3x width of remaining lamellae; 5th lamellae with lobe extending along base of lamellae 3 and 4; respiratory suture begins at the gap between 1st lamellae and the apex of the lobe from the 6th lamellae, and continues between lamellae 3 and 4.

Type Material.—Holotype [4th stage larvae]: MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LP], coll. R. Sam. **Other material examined**.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LP], 13 June 2006 [L], 6 January 2007 [LP]; Manjakandriana, 1958, Ambatobaona; *Fianarantsoa*: Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January 2007, Riambavy, Riandahy, Zomandao [LP]; Ambalavao,

1958, Vakona R. Specimens from Manjakandriana and Ambalavao collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Eupaulianina telofantsy* collected in eastern-central and south-central rainforests in both large and small streams. It is sympatric with *E. marobotsin*, *E. tsilobe*, *E. silva*, *E pamela*, *E. tandroka*, *E rivalis*, *P. lehibe*, and *P. umbra*.

Bionomics.—Larvae and pupae collected in fast flowing water in both small and larger streams, mainly along stream margins where water flow was less then 5cm deep across surface of substrate.

Etymology.—From Malagasy for "three sharp nails" (telo-three, fantsy-sharp, curved nail), in reference to the fact that the larva has three large spines dorsally.

Eupaulianina tsilobe Sam and Courtney, new species (description based on pharate adult) (Figs. 15, 30, 92, 94, 96, 98, 147)

Syn: Species I (Stuckenberg) 1958

Species I Stuckenberg 1958: 163-166. Zwick 1977: 27 (review of morphology and phylogenetics). Arens 1998: 84, 109-110.

Diagnosis.—medium blephariceridid. Larva: Cranial sclerites dark brown, posterior margin produced posteriorly in median line into a broad lobe. AM with tubal tubercles tipped with three sharp spine like sensillum. Pupa: Respiratory organ kidney-shaped with 6 lamellae; 1st lamellae large, swollen, pointed at apex, remaining lamellae separate. Papillae with 2-3 slender, pale, leaflet-like projections arises from papillae posterior with series of spinelets at base. Adult: Dorsal paramere elongate with distinct rounded lobes at lateral apices; lateral aedeagal filaments enter ventral parameres.

Description.—Larva (Figs. 15, 92): Measurements, instar III (n = 1): Total length 3.6mm, cranial width 1.8mm, antennal segment 0.22mm; instar IV (n = 18): Total length 4.1mm (1.8-4.9), cranial width 1.16mm (1.1-1.3), basal antennal segment 0.05mm (0.03-0.08), apical antennal segment 0.08mm (0.04-0.11). Larva slender with elongate spines across body. Cranial sclerites dark brown, posterior margin produced posteriorly in median line into a broad lobe; lobe margin curves around AD, making contact with them at anterior points; ecdysial line with short stem; posterior margin of frontoclypeal apodeme does not reach posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed at midline. Coloration: Body uniformly light brown. Raised plates brown; spines piceous. Lateral lobes with prolegs visible dorsally. Chaetotaxy: Larval integument strigate on both abdominal segments and intercalaries; body with a series of elongate spines on raised, glabrous plates, spines with long, glabrous base, tipped with pointed, elongate striated sensillum; AL a single elongated, sub rectangular plate with long axis vertical, produced into a small tubercle at lower end; AD large, occupying the recess on each side of lobe of head capsule, composed of two spines, the inner one 3x length of outer, both curved and reclinate; M smooth, without spines; LP composed of single long spine, curved inwardly; AM composed of three large spines set evenly on large ovoid plate, spines curved and proclinate; Both PM and PP absent; R subquadrate with transverse furrow across middle, a single erect spine set in each corner ; S composed of two single spines, each set on lateral ends of narrow ovoid plate; T composed of two erect, small spines; anal division with several spines directed posteriorly along posterior margin; intercalary segments with 2 small spines on lateral corners; prolegs with several small spines scattered dorsally and dorsolaterally, tipped with several projecting tubercles.

Pupa (Figs. 30, 94, 96, 98): Measurements, male (n = 1): Total length 3.8mm, width 2.2mm; female (n = 5): Total length 4.74mm (4.5-4.85), width 2.58mm (2.45-2.7); undetermined (n = 5): Total length 3.25mm (1.6-3.8), width 2.0mm (1.6-2.2). Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Integument: Dorsal papillae present, uniformly dispersed across all sclerites and tergites. Papillae well developed, glabrous; ovoid, with scalloped margin; 2-3 slender, pale, leaflet-like projections arises from papillae posterior with series of spinelets at base. Cuticle between papillae rugose, uniform in pigmentation. Respiratory organ dark brown, kidney-shaped with 6 lamellae; 1st lamellae large, swollen, pointed at apex; lamellae 4-6 appear as swellings with distinct separation; lamellae 2 and 3 merged together, with small separation at their base; remainder of organ is large swelling, pointed at outermost apex; lamellae 6 extends around base of lamellae 3-5, ending at the separation between lamellae 2 and 3; distinct groove starts at apex of lamellae 6, continuing forward through ½ of 1st lamellae.

Adult male (Fig.147): Size: Small. Measurements (n = 3): Total length 3.56 mm, wing length 5.63mm, wing width 1.94mm.

Leg – Segment Lengths:

		foreleg	midleg	hindleg
femur		4.56 (3.75-5.0)	5.76 (5.0-6.13)	6.44 (5.57-6.86)
tibia		5.16 (4.38-5.57)	4.52 (3.81-4.94)	6.86 (5.94-7.81)
tarsus	1	2.38 (2.06-2.56)	2.38 (2.0-2.56)	1.4 (1.18-1.68)
	2	0.72 (0.63-0.81)	0.75 (0.63-0.81)	0.47 (0.44-0.50)
	3	0.31	0.33 (0.31-0.38)	0.28 (0.19-0.38)
	4	0.16 (0.13-0.19)	0.19	0.22 (0.13-0.31)
	5	0.22 (0.19-0.25)	0.23 (0.19-0.31)	0.22 (0.19-0.25)

Head Structure: Eyes dichoptic, interoccular distance = 0.21mm (0.19 – 0.22); eyes not divided, ommatidia subequal. Clypeus length/width = 2.5. Proboscis about 0.5x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 0.8 - 1.1 - 1.9. Antennal segments cylindrical; apical segment subequal to pentultimate segment; scape and pedicel brown with prominent setiforms, remaining flagellomeres brown and setose. Chaetotaxy: Post-genals numerous, clypeals numerous.

Thorax: Wing spur long, 0.66x length of r-m; Cu and M_4 fused for short distance, anal vein meets does not wing margin. Last middle tarsomere 1.2x penultimate segment. Leg segment proportions: foreleg—34:38:18:5:2:1:2, midleg—41:32:17:5:2:1:2, hindleg—41:43:8:3:2:1:1. Chaetotaxy: Thorax glabrous except for dense clumps of setae on the lateral ends of scutellum.

Coloration: Frons and clypeus pale brown; occiput dark; scutum and scutellum chestnut brown.

Terminalia (Fig. 147): Gonostyles curved sharply towards midline, with abundant setiform sensilla; gonocoxite long, with abundant setiform sensilla along lateral boundary; gonocoxal lobes elongate, rounded at apex, flexed inward; ventral bridge appears ovoid ventrally, with two invaginations posteriorly; ventral plate appears as half circle with lobes at lateral apices; dorsal paramere elongate with distinct rounded lobes at lateral apices, slight rounding along midline; vesica hourglass-shaped; ventral parameres rounded distally at base, curving inward, slightly lobed at apex; lateral aedeagal filaments enter ventral parameres, medial aedeagal filament encased in slender tube, bulbous at base; apodeme lobed, with ridge down median line.

Adult female: unknown.

Type Material.—Holotype [pharate adult male]: MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, 14 October, 2004, coll. R. Sam. **Other material examined**.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LPA], 13 June 2006 [L], 6 January 2007 [LP]; *Toamasina*: "Perinet" now known as Andasibe, next to Mantadia National Park, December 1955; Vohimana Reserve, S18°56.389' E48°30.999', 29 June 2006, Relais du Naturaliste, Iasina River, [L]; Vohimana Reserve, S18°55.273' E48°30.77', 29 June 2006, Relais du Naturaliste, Vohimana R @ xing [L]; Vohimana Reserve, S18°55.362' E48°30.72', 29 June 2006, Vohimana R., falls next to village [L]; Vohimana Reserve, S18°55.462' E48°30.58', 29 June 2006, Tsat sahina flowing into Vohimana R. [LP]; Vohimana Reserve, 03 Jan 2007, Village de Chercher/Nursery [LP]; Sahatandra R., S18°54.384' E48°28.83', trib. into river [L]. Specimens from Andasibe collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Eupaulianina tsilobe* seems to be limited to central Madagascar, in the highlands (AMSR) and rainforest areas (VOH). It is sympatric with *P. umbra*, *E. alexanderi*, *E. rivalis*, *E. pamela*, *E. silva*, *E. marobotsin*, *E. tandroka*, and *E. telofantsy*.

Bionomics.—Adults collected in October (AMSR) and January in VOH around small shaded streams. Larvae and pupae collected in fast flowing water in both small and larger streams, as well where the main flow from waterfalls forcefully strike the rocks below.

Etymology.—From Malagasy for "large spine" (tsilobe), in reference to the large dorsal spines on the larva.

Eupaulianina fantsona Sam, new species (Figs. 148, 149)

Diagnosis.—Blephariceridae. Adult: Ventral plate ovoid, hollow center; lateral aedeagal filaments enter ventral parameres, medial aedeagal filament encased in slender straight tube, rounded at apex

Larva: Unknown.

Pupa: Unknown.

Adult female: Unknown.

Adult male (Figs. 148, 149): Size: medium. Measurements (n = 1): Total length 3.0mm, wing length 4.56mm, width 1.75mm.

Leg –

Segment

Lengths:		foreleg	midleg	hindleg
femur		3.13	3.75	4.31
tibia		3.49	3.13	4.63
tarsus	1	1.56	1.56	1.06
	2	0.50	0.63	0.31
	3	0.19	0.25	0.19
	4	0.13	0.13	0.13
	5	0.19	0.19	0.19

Head. Eyes dichoptic, interoccular distance = 0.11mm; eyes not divided, ommatidia subequal. Clypeus length/width = 2.25. Proboscis about 1.0x head width; mandible absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0 - 1.2 - 1.6 - 2.2. Antennal segments cylindrical; apical segment subequal to pentultimate segment; scape and pedicel brown with prominent setiforms, remaining flagellomeres brown and setose. Chaetotaxy: Post-genals numerous, clypeals numerous.

Thorax. Wing spur short, 0.3x length of r-m; Cu_1 and M_4 fused for short distance, anal vein does not meet wing margin. Last middle tarsomere 1.5X penultimate segment. Leg segment proportions: foreleg—35:37:17:6:2:1:2, midleg—39:32:17:7:3:1:2, hindleg—39:42:10:3:2:1:2. Chaetotaxy: Thorax glabrous except for dense clumps of setae on the lateral ends of scutellum.

Coloration. Frons and clypeus brown; occiput dark; scutum and scutellum dark brown.

Terminalia. Gonostyles curved sharply towards midline, with abundant setiform sensilla; gonocoxite broad, with abundant setiform sensilla along lateral boundary; gonocoxal lobes broad at base, then elongate, rounded at apex, slightly deflexed; ventral bridge appears ovoid ventrally, with two slight invaginations anteriorly; ventral plate ovoid, hollow center; dorsal paramere elongate with inward curve along apex of lateral edge and lobed at apical corners; vesica hourglass-shaped; ventral parameres rounded distally at base, curving inward, slightly flared at apex; lateral aedeagal filaments enter ventral parameres, medial aedeagal filament encased in slender straight tube, rounded at apex; apodeme lobed, with ridge down median line.

Adult female: Unknown.

Type Material.—Holotype [adult male]: MADAGASCAR: *Toamasina*: Vohimana Reserve, 03 Jan 2007, coll. R. Sam. **Other material examined**.—MADAGASCAR: *Toamasina*: Vohimana Reserve, 03 Jan 2007, Village de Chercher/Nursery [A]; All material collected by R. Sam.

Distribution.—*Eupaulianina fantsona* seems to be limited to east central Madagascar, in the rainforest areas (VOH). It is sympatric with *P. umbra*, *E. alexanderi*, *E. rivalis*, *E. pamela*, *E. silva*, *E. marobotsin*, *E. tandroka*, and *E. telofantsy*.

Bionomics.—Adults January in VOH around small shaded streams. Larvae and pupae collected in fast flowing water in both small and larger streams.

Etymology.—From Malagasy for "tube" or pipe (fantsona), in reference to the medial filament being encased in slender straight tube .

Eupaulianina marobotsin Sam (Figs. 34, 119-124)

Species E Paulian 1953: 437. Stuckenberg 1958: 111.

Diagnosis. —Small blephariceridid. Larva: AM with cluster of rounded tubercles arranged in a alternating pattern; gills may or may not be directed laterally rather then ventrally. Pupa: Respiratory consists of 7 swellings with distinct space between, five of which a round lobes; glabrous papillae with single, elongo-elliptoid projection extended from posterior margin on a slender stem from group of spinelets.

Description.—Larva (Figs. 34, 119-121): Measurements, instar II (n = 7): Total length 1.48mm (1.27-1.57), cranial width 0.22mm (0.19-0.33), antennal segment 0.15 (0.11-0.33); instar III (n = 15): Total length 2.63mm (1.8-3.24), cranial width 0.3 (0.27-0.33), antennal segment 0.21 (0.18-0.25); instar IV (n = 20): Total length 3.79mm (2.38-4.94)), cranial width 0.45mm (0.39-0.50), basal antennal segment 0.13mm (0.11-0.16), apical antennal signal 0.2mm (0.19-0.26). Cranial sclerites dark brown with black margin posteriorly; ecdysial line with short stem line; frontoclypeal apotome extend nearly to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary

segments narrowed at midline. Prolegs of cephalothorax subcylindrical, blunt apically; prolegs of anal division subcylindrical, narrow, blunt apically; remaining prolegs large, conical, tapered apically, blunt apically. Clear division between last abdominal segment and anal segment; Coloration: uniformly dark brown. Lateral lobes with prolegs visible dorsally, light brown. Chaetotaxy: AD stout conical tubercle; AL composed of several conical piceous tubercles in a vertical line; M composed of a plate with 5-6 rounded tubercles, darkly tipped, arranged in an alternating posterior-anterior pattern; LP light brown with piceous tip, with yellow-brown spine laterally, tipped with both black and yellow-brown spines; AM composed of a plate with 5-6 rounded tubercles, darkly tipped, arranged in an alternating posterior-anterior pattern; PM same structure as AM; PP composed of a rounded, darkly tipped tubercle, closely associated with PM; R composed of two plates, aligned together to form trapezoid, pattern of tubercles similar to AM and PM; S composed of small plate, aligned to posterior margin of R, two tubercles laterally; T present as two single tubercles. Lateral prolegs with large yellow-brown spines and dense apical patch of long, yellow setae; anal prolegs with single large yellow-brown spines and dense apical patch of long, light brown setae

Pupa (Fig. 34, 122-124): Measurements, male (n = 1): Total length 3.0mm, width 1.68mm; female (n = 3): Total length 3.38 (3.32-3.52), total width 1.77mm (1.68-1.88); undetermined: (n =1): Total length 3.32mm, total width 1.6mm. Cuticle dark brown. Body roughly ovoid, narrowing at alar sclerites; third abdominal segment swollen at lateral ends. Anal segment truncated along posterior margin. Papillae present, uniformly dispersed across cephalic sclerite, branchial tergites, metatergite, abdominal tergites, and anal tergite; absent on alar sclerite. Body constricted at anterior portion of alar sclerite. Papillae well developed,

glabrous; ovoid; small clump of spinelets present along posterior margin; single, elongo-elliptoid projection extended from posterior margin on a slender stem from group of spinelets. Papillae cuticle between smooth, uniform in pigmentation. Respiratory lamellae dark brown. Respiratory organs elongated transversely. Respiratory lamellae light brown. 7 distinct lamellae as swellings with distinct space between. Lamellae 1 narrow ovoid structure beginning at outer point of organ, curving across anterior margin, around lamella 2, towards edge of lamellae 3 and 4; lamellae 2 round, placed within curve of lamella 1; lamellae 3-6 round, clustered in square within the curve of lamellae 7; inner 7th lamella J-shaped, round at interior margin, curving around lamellae 3-6 to reach posterior margin of lamella 2.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage Larva]: MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [L], coll. R. Sam

Other Material examined.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [L], 13 June 2006 [L], 6 January 2007 [L]; *Toamasina*: Analamazaotra Special Reserve, S18° 55.717' E48° 25.467', 16 June 2006 [L]; Maroansetra, 1958, Ambodivohangy; Maroansetra, 1958, Mahalevona, Ambohitsitondroina; *Antsiranana*: Montagne d'Ambre, 1958, Rousettes Forest Station. Specimens from Maroansetra and Montagne d'Ambre collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Eupaulianina marobotsin* is located in the north mountains, central highlands and eastern rainforests. It is sympatric with several species, including *P. umbra*, *E. alexanderi*, *E. rivalis*, *E. pamela*, *E. silva*, *E tsilobe*, *E. telofantsy*, and *E. tandroka*.

Bionomics.— *Eupaulianina marobotsin* larvae can be collected year round, which is common for Malagasy blepharicerids. Because this specimen has not been associated with an adult species, further speculation regarding life history is limited. It is generally collected in small, shaded streams, though it has also been collected in a small cave in MNP.

Etymology.—From Malagasy for "many bumps" (marobotsin), in reference to the presence of many bump-like structures located on the dorsal plates of the larva.

Eupaulianina pamela (Stuckenberg) comb. nov. (Figs. 9, 10, 27, 61-74)

Syn: *Paulianina pamela* Stuckenberg 1958

Eupaulianina pamela Stuckenberg 1958: 150-156. Zwick 1977:10 (review of morphology and phylogenetics). Arens 1998: 84, 92-96, 106-110, 112.

Diagnosis.— Small blepharicerid. Larva: with two distinct morphs; conical protuberances tipped with pointed sensilla, either stout or long and tapered. Pupa: Respiratory lamellae dark brown, small and pear-shaped. Five lamellae develop as raised swellings with evident space between. Adult: Wing spur very short; male with lanceolate aedeagal filaments.

Description.—Larva (Figs. 9, 10, 61-70): Measurements, instar I (n=1): Total length 1.48mm, cranial width 0.16mm, antennal segment 0.11mm; instar II (n=3): Total length 3.3mm (2.9-3.5), cranial width 0.30mm (0.29-0.33), antennal segment 0.14mm (0.13-0.14); instar III (n=5): Total length 2.8mm (2.1-3.5), cranial width 0.34mm (0.31-0.34), antennal segment 0.16mm (0.15-0.17); instar IV (n=20): Total length 4.1mm (3.3-5.4), cranial width 0.50mm (0.43-0.54), basal antennal segments 0.11mm (0.08-0.13), apical antennal segment 0.15mm (0.11-0.17). Larva slender with stout spines across body. Cranial sclerites dark

brown; ecdysial line with short stem; posterior margin of frontoclypeal apodeme does not reach posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed at midline. Coloration: Uniformly light brown medially, with lateral lobes becoming pale yellow brown apically. Lateral lobes with prolegs visible dorsally. Chaetotaxy: Larval integument strigate on abdominal segments, glabrous on intercalaries; microsculpture on raised plates, smooth with evenly spaced, dome-like microstructure; AL 3-4 stout conical structures with a single stout striated sensilla arising dorsally; AD paired stout conical structures with single striated sensilla dorsally; M elevated rounded plate, four conical protuberances arranged evenly in lateral line across plate, each with apical single stout, striated, conical sensilla dorsally; LP extended smooth conical projection tipped with single stout, striated, conical sensilla, often with 2-3 additional similar projections lateromedially; AM elevated rounded plate, three conical protuberances arranged evenly in lateral line across plate, each with apical single stout, striated, conical sensilla, similar to M, however conical protuberances 2x size of those on M; PM elevated rounded plate, two conical protuberances arranged at lateral ends of plate, each with apical single stout, striated, conical sensilla, plate ½ overall size of AM; PP single conical protuberance with a single stout striated sensilla arising dorsally; R elevated trapezoidal plate with five conical protuberances, three larger arranged in lateral line across the anterior of plate, two smaller arranged in at posterior corners of plate, each protuberance with apical single stout, striated, conical sensilla; S elevated rounded plate, two conical protuberances at lateral ends of plate, each protuberance smooth towards apex, with apical single stout, striated, conical sensilla; T smooth with two small conical protuberances on each lateral edge, both with

apical single stout, striated, conical sensilla; prolegs with several sensillua scattered dorsally and dorsolaterally, tipped with dense apical patch of long, yellow setae.

Pupa (Figs. 27, 71-74): Measurements, male (n=10): Total length 2.8mm (2.6-3.1), width 1.5mm (1.3-1.6); female (n=11): Total length 3.3mm (2.3-3.5), width 1.6mm (1.1-1.9mm). Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Anterior marked with broad transverse ridge. Integument: Dorsal papillae present, uniformly dispersed across all branchial sclerite, cephalic sclerite, abdominal tergites, and anal tergite; absent on alar sclerite. Papillae glabrous, ovoid with small clump of spinelets along posterior half; single globose projection extended posteriorly from base of spinelets; cuticle between papillae homogenous, without patterning. Respiratory lamellae dark brown, small and pear-shaped. Five lamellae develop as raised swellings with evident space between. Lamella 5 is 2x as long as lamella 1; elongated; a gradually narrowing process extending halfway down the posterior margin of respiratory organ. Lamellae 2-4 subequal, 0.6x length of inner lamella. Small notch just to the outside of 2nd lamellae. Respiratory suture short; begins at notch running directly between lamellae 3 and 4.

Adult male (Based on Stuckenberg 1958) Size: medium. Wing length = 4.13mm; Wing width = 1.5mm.

Head. Labrum short, subequal in length to basal flagellar segment. Palpi with 5 palpomeres, distal 4 segment proportions from base to apex 15.2 - 11 - 8.0 - 12. Antennal segments with long fine macrotrichia; apical segment subequal in length to penultimate segment.

Thorax. Wing spur 0.5x r-m; M_3 curved, not continued as row of microtrichia; anal vein incomplete; M_4 and Cu not fused for any distance. Last tarsomere on middle leg 0.6x length of penultimate segment.

Terminalia. (Fig. 142) Gonostyles curved sharply towards midline, with abundant setiform sensilla. Gonocoxite broad, with abundant setiform sensilla along lateral boundary. Gonocoxal lobes elongate, rounded at apex, flexed inward. Ventral bridge present as subcrescentic structure. Dorsal paramere broadly rounded, with small outward lobe along the midline. Vesica mushroom-shaped. Aedeagal filaments lanceolate, lateral filaments projected at angles from central filament. Ventral parameres curve around aedeagal filaments, not meeting at apex. Apodeme lobed, with ridge down median line.

Adult female: (Based on Stuckenberg 1958): Size: medium. Wing length = 4.81mm; Wing width = 1.75mm.

Terminalia. Eighth sternite with elongate window at base of median fold. Hypogynial valve broad basally, narrowed apically into two narrowed, valves; individual valves broad basaly, rounded apically. Accessory gland elongate. Spermathecae 3 in number, spherical, simple, with short annular necks; Duct walls with spine-like projections and thickenings. Chaetotaxy: Sparse setae across sternite VIII; hypogynial plate and valves pruinose.

Type Material.—Holotype [adult male, pinned]: MADAGASCAR: *Toamasina*: "Perinet", December 1955, coll. B. Stuckenberg. Specimens collected from a small stream "few miles east" of "Perinet", now known as the village of Andasibe. "Stream flowed into larger river that flowed past the village". "Site 800m from large sawmill". [IRSM]. Allotype [1 female A, pinned]: same date at holotype [IRSM]. Paratypes [2 male A (pinned), 2 male A

(dissected), 1 female A (dissected)]: same data as holotype. Paratypes deposited in IRSM and NM.

Other material examined.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LPA], 13 June 2006 [L], 6 January 2007 [LPA]; *Toamasina*: Mangabe, Maroansetra 1958; *Fianarantsoa*: Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January 2007, Riambavy Falls, Riandahy Falls, Zomandao R. [LPA]; Ranomafana National Park, S21°15.86' E47° 24.69', 10-11 October 2004 Sacre Roa and trib. [L]; Ranomafana, S21°15.232' E47°27.447', 12 October 2004, village waterworks [L]. Specimens from Maroansetra collected by B. Stuckenberg; all other specimens collected by R. Sam.

Distribution.—*Eupaulianina pamela* is widely distributed across Madagascar (spans three provinces), though at a limited number of sites. The species is sympatric with several species, including *P. umbra*, *E. rivalis*, *E. silva*, *E. tsilobe*, *E. telofantsy*, *E. tandroka*, *E. marobotsin*, and *E. botsimpatsy*. Larvae and pupae greatly outnumber *Paulianina* s. s. when collected on the same rock.

Bionomics.—*Eupaulianina pamela* seems to be active all year, with peak adult activity occurring December-February. Stream conditions were similar to those described for *P. umbra*. Adults taken by Stuckenberg were collected mainly in morning hours, flying in small groups under large rock overhangs close to waterfalls. Larvae and pupae originally described by Stuckenberg were collected in larger streams, on more submerged rocks, similar to the habitat of *P. ingens*. However, this was not the case on 2004-2007 collecting trips, during which larvae were collected in a wide variety of habitats. Larvae and pupae were most abundant on rocks at the base of waterfalls under very strong flows of water. Larvae were

also collected on moss-covered splash zones of the waterfall and madicolous habitats behind waterfall. Larval *Eupaulianina pamela* demonstrate several morphotypes (ie. short dorsal spines to long dorsal curved spines), the only Malagasy larvae to display this trend. Specimens collected at ANP were larger in size than other specimens.

Eupaulianina rivalis (Stuckenberg) comb. nov. (Figs. 13, 83-90, 145)

Syn: *Paulianina rivalis* Stuckenberg 1958

Eupaulianina rivalis Stuckenberg 1958: 157-163. Zwick 1977: 7-10 (review of morphology and phylogenetics). Arens 1998: 84, 96-99, 107, 109-112.

Diagnosis.—Small blephariceridae. Larva: Chaetotaxy consists of round, smooth, flat-topped microsculpturing evenly dispersed across plates covering abdominal segments 1-6 and anal segment. Pupa: Papillae glabrous; ovoid with scalloped edges; small clump of spinelets present along posterior margin; single globose projection extended posteriorly from base of spinelets. Adult: Wing spur greatly reduced; aedeagal filaments lanceolate, flattened; ventral bridge elaborate with long extended thickened lateral arms; apodeme large and flared.

Description.—Larva (Fig 13, 83-87): Measurements, instar II (n = 1): Total length 1.48mm, cranial width 0.22mm, antennal segments 0.12mm; instar III (n = 10): Total length 1.9mm (1.48 – 2.47), cranial width 0.37mm, antennal segment 0.2mm; instar IV (n = 9): Total length 3.1mm (2.4 – 4.2), cranial width 0.55mm (0.51 – 0.59), basal antennal segment 0.46mm (0.44-0.48), apical antennal segment 0.46mm (0.44-0.48). Larvae small and stout. Cranial sclerites light brown with light patches along black posterior margin; Ecdysial stem line with short stem line, frontoclypeal apotome extend nearly to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments not narrowed

at midline. Coloration variable: trunk uniformly light brown, with lateral lobes becoming pale yellow brown apically; abdominal segments dark brown, ringed with a thin black line. Lateral lobes with prolegs visible dorsally. Chaetotaxy: Round, smooth, flat-topped microsculpturing evenly dispersed across plates covering abdominal segments 1-6 and anal segment; cuticle between tubercles rugrose; microsculpturing may look granular under light microscope. AD composed of long transversely narrow plate, more narrow along median line; AL with small round plate towards medial side, with two conical tubercles laterally; M long, narrow ovular shape extending across the posterior side of cephalothorax; AM long, narrow ovular shape extending across anterior of abdominal segments, three tiny, stout, conical sensillum along anterior border, one placed at each lateral corner and one at midline; LP raised, smooth towards apex, tipped with single, stout conical sensilla; PM long, narrow ovular shape extending across the posterior half of abdominal segments, approximately ½ longitudinal width of AM, two tiny, stout, conical sensillum set centrally, equidistant from each other from midline; PP absent; R trapezoidal in shape, with rounded edges; S long, narrow, with scalloped edges; T narrow posterior border of anal segment; intercalary segments with 2 small spines on lateral corners; prolegs with several small spines scattered dorsally and dorsolaterally, tipped with dense apical patch of long, yellow setae.

Pupa (Figs. 88-90): Measurements based on Stuckenberg 1958: Length 3.63mm. Cuticle dark brown. Body roughly ovoid, dorsoventrally compressed. Integument: Dorsal papillae present, uniformly dispersed across all branchial sclerite, cephalic sclerite, abdominal tergites, and anal tergite; absent on alar sclerite. Papillae well developed, glabrous; ovoid with scalloped edges; small clump of spinelets present along posterior margin; single globose projection extended posteriorly from base of spinelets. Cuticle

between papillae faintly rugose, uniform in pigmentation. Respiratory lamellae dark brown. Five swellings placed closely next to each other with slight valleys between; 5th outermost lamella 3x the size of the remaining lamella; 5th lamella wrapped around posterior end of 3rd and 4th lamellae; respiratory suture forms at the apex of the 5th lamella, continuing between lamellae 3 and 4.

Adult male. (Fig. 145) (Based on Stuckenberg 1958): Size: small. Measurements: Wing length 5.62mm, width 2.2mm.

Head. Labrum short, 1.6x length of basal flagellar segment. Palpi with 5 palpomeres, distal 4 segment proportions from base to apex 16 - 11 - 7.5 - 12.5. Antennal segments with long fine macrotrichia; apical segment subequal in length to penultimate segment.

Thorax. Spur vestigial, greatly reduced; anal vein incomplete, continued by row of microtrichia; Rs before r-m; last tarsomere of middle leg 2x pentultimate segment.

Terminalia. (Fig. 145) Gonostyles curved broadly posteriorly, slightly flexed inwards, with abundant setiform sensilla; gonocoxite broad, with abundant setiform sensilla along lateral boundary; gonocoxal lobes set on subquadrate base, elongate, pointed at apex, directed horizontally toward midline; ventral bridge elaborate, encloses a semiorbicular space with long broad lateral arms that are thickened in the middle, curving posteriorly alongside parameres and filaments; ventral plate inverted narrow trapezoid; dorsal paramere appears as two trapezoidal shapes, overlapping along midline; vesica elongate; aedeagal filaments lanceolate, lateral filaments projected at angles from central filament; ventral parameres curved, with apices almost meeting; apodeme large and flared, with distinct ridge down median line.

Adult female: (Based on Stuckenberg 1958) Hypogynial valve elongate, slender with apical lobes slender, very narrowed apically. Accessory gland narrow, elongate, extends past spermathecae. Spermathecae 3 in number simple, ovoid. Ducts narrow, covered with fine ridges.

Type Material.—Holotype [adult male]: MADAGASCAR: *Toamasina*: "Perinet", December 1955, coll. B. Stuckenberg. Specimens collected from a "small stream 5k east" of "Perinet", now known as the village of Andasibe. "Stream flowed into larger river that flowed past the village". "Site 800m from large sawmill". [IRSM]. Allotype [adult female, pinned]: same data a holotype [IRSM]. Paratypes: same data as holotype, [numerous larvae and pupae]. Paratypes deposited in both IRSM and NM.

Other material examined.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [LPA], 13 June 2006 [L], 6 January 2007 [LPA]; *Toamasina*: Mantadia National Park, S18°49.717' E48°26.417', 16 October 2004 [L], 15 June 2006 [L]; Analamazaotra Special Reserve, S18° 55.717' E48° 25.467', 16 June 2006 [L]; Maroansetra, 1958, Ambodivohangy**;** Antanambe, N. of Ivontaka, 1958; *Fianarantsoa*: Ranomafana National Park, S21°15.86' E47° 24.69', 10-11 October 2004 Sacre Roa and trib. [L]; Ranomafana, S21°15.232' E47°27.447', 12 October 2004, village waterworks [L]. Ambalavao/Ambaravarandanitra Mt. 1958**.** Specimens from Maroansetra, Antanambe, and Ambalavao collected by B. Stuckenberg; all other material collected by R. Sam.

Distribution.—*Eupaulianina rivalis* appears to have a limited distribution, not being collected north of Maroansetra or south of RNP. Its range extends westward to AMSR. It is

sympatric with many species, including *P. umbra*, *E. alexanderi*, *E. pamela*, *E silva*, *E. tsilobe*, *E. tandroka*, *E. marobotsin*, and *E. telofantsy*.

Bionomics.—Larvae and pupae originally described by Stuckenberg as collected in larger streams, on more submerged rocks, similar to *P. ingens*. However, this did not seem to be the strict case in the 2004-2007 collecting trips, with larvae being collected in a wide variety of habitats, generally found on rock surfaces covered with a fairly thin flow of water.

Eupaulianina tandroka Sam, new species (Figs. 24, 107-112)

Diagnosis.— Small blepharicerid. Larva: Superficially similar to E. rivalis; dorsal microsculpturing dome-like; lateral edges of AM plates flared into large cone-shaped tipped with single stout conical sensillum.

Description.—Larva (Figs. 24, 107-112): Measurements, instar III (n = 6), total length 1.63mm (1.18 – 1.78), cranial width 0.31mm (0.3 – 0.35), antennal segments 0.17 (0.12 – 0.20); instar IV (n = 15), total length 2.75mm (2.20 – 2.47), cranial width 0.43mm (0.37 – 0.49), antennal segment. Larva small and stout. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Chaetotaxy: Dome-like microsculpturing evenly dispersed across abdominal and intercalary segments; cuticle between tubercles rugrose; microsculpturing may look superficially similar that of *E. rivalis* under light microscope; cuticles dark brown, ringed with a thin black line. AD composed of long transversely narrow, more narrow along median line; AL with small round plate towards medial side, with two conical tubercles laterally; M long, narrow ovular shape extending across the posterior side of cephalothorax; AM long, narrow ovular shape extending across the anterior abdominal segments, single tiny, stout, conical sensillum along midline at

anterior border; lateral edges of plate each with single large cone-shaped flared areas tipped with stout conical sensillum; LP raised, smooth towards apex, tipped with single, stout conical sensilla; PM long, narrow ovular shape extending across the posterior half of abdominal segments, approximately ½ longitudinal width of AM, two tiny, stout, conical sensillum set centrally, equidistant from each other from midline; PP absent; R trapezoidal in shape, with rounded edges; S long, narrow, with scalloped edges; T narrow posterior border of anal segment.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th instar Larva]: MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004, coll. R. Sam. **Other material examined**.—MADAGASCAR: *Antananarivo*: Ambohitantely Special Reserve, S18°11.88' E47°16.89', 14 October 2004 [L], 13 June 2006 [L], 6 January 2007 [L]; *Toamasina*: Analamazaotra Special Reserve, S18° 55.717' E48° 25.467', 16 June 2006 [L]; All specimens collected by R. Sam.

Distribution.—*Eupaulianina tandroka* seems to have a distribution limited to central Madagascar, though collected both in the highlands (AMSR) and the eastern rainforest ANSR and MNP). It is sympatric with *P. umbra*, *E. alexanderi*, *E. rivalis*, *E. pamela*, *E. silva*, *E. tsilobe*, *E. marobotsin*, and *E. telofantsy*.

Bionomics.— Larvae generally collected in small, shaded streams. Larvae also collected in a small cave located in MSP, where there was a small film of water flowing over the cave walls.

Etymology.—From Malagasy for “zebu horn” (tandroka), in reference to the large horn-like structures located on the dorsum of the larva.

Eupaulianina borivody Sam and Courtney, new species (Figs. 17, 116-118)

Diagnosis.—Medium blephariceridae. Larva: Chaetotaxy present as small piceous, conical tubercles. Anal division appears roundly swollen. Pupa: Lamellae present as six swellings, with lamellae one and two mostly merged and lamellae five and six mostly merged.

Description.—Larva (Figs. 17): Measurements, instar IV (n = 13): Total length 4.9mm (3.3-6.3), cranial width 0.49mm (0.44-0.54), basal antennal segment 0.16mm (0.12-0.17), apical antennal segment 0.2mm (0.17-0.22). Cranial sclerites dark brown with black margin posteriorly; ecdysial line with short stem line; frontoclypeal apotome extend nearly to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Coloration: Body uniformly brownish yellow. Raised dorsal plates light brown, similar in color to cranial sclerites. Lateral lobes with prolegs visible dorsally. Prolegs of cephalothorax tapered, narrowing apically, blunt apically; prolegs of anal division subcylindrical, blunt apically; remaining prolegs large, conical, tapered apically, rounded apically. Anal division appears swollen outwardly. Chaetotaxy: AL composed of three piceous, small, conical tubercles in vertical row, with upper tubercle twice as far from middle tubercle then basal tubercle; AD consists of 2-3 conical, piceous tubercles, slightly larger then AL; LP yellowish-brown, stout, conical; M present as subrectangular, smooth, light brown plate with two piceous, small, conical tubercles; AM present as subrectangular, smooth, light brown plate with three piceous, conical tubercles, larger then the tubercles on

M, though still small; PM present as subrectangular, smooth, light brown plate with two piceous, small, conical tubercles; PP present as small, piceous, conical tubercle; R present as two subrectagular plates, similar in size and shape to AM and PM, respectively each bearing two small, piceous, conical tubercles; S present as single subrectangular smooth, light brown plate bearing two small, piceous, conical tubercles; T absent. Clear division between last abdominal segment and anal segment present, but faint; Prolegs with large yellow spines and dense apical patch of long, yellow setae.

Pupa (Figs. 116-118): Pupal specimen only anterior section, no measurements. Integument: Dorsal papillae present, uniformly dispersed across cephalic sclerite, abdominal tergites, and anal tergite; absent from the lateral ends of the metatergite sclerite, absent in area between medial anterior of alar sclerite; absent on alar sclerite. Papillae well developed, glabrous; ovoid, smooth along margin; small clump of spinelets present along posterior margin; single, elongo-elliptoid projection extended from posterior margin on a slender stem from group of spinelets. Respiratory lamellae appear as 6 swellings with little to no space between. Lamellae 1-2 partially merged. Lamellae five and six almost completely merged. Limited, shallow space between lamellae two and three, three and four, and four and five.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage Larva]: Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January 2007, coll. R. Sam. **Other Material examined**.—MADAGASCAR: *Antananarivo*: Tsiafajavona Peak (elev. 2600m), 1955; *Toamasina*: Maroansetra, 1958, Mangabe**;** *Fianarantsoa*: Andringitra National Park, S22° 08.73' E46° 53.46', 10-11 January 2007, Riambavy, Riandahy, Zomandao [L]; Ambalavao, 1958, nr.

Antanifotsy; Soaindrana Plateau. Specimens from Tsiafajavona, Maroansetra, Ambalavao, and Soaindrana collected by B. Stuckenberg; specimens from Andringitra collected R. Sam.

Distribution.—*Eupaulianina borivody* is limited to south and central Madagascar, near Antananarivo extending eastward to the eastern rainforests and south to ANP. It is sympatric with *P. ingens*, *E. alexanderi*, *E. pamela*, *E. silva*, and *E. telofantsy*.

Bionomics.—Because collection has been so limited and only extends to larvae, it is difficult to draw conclusions regarding the species' behavior. Originally collected in a stream located on Mount Tsiafajavona located north-east of Antananarivo, very few specimens are collected from each site. Larvae have only been collected during January, which is different from other Malagasy blephariceridsm, which are usually collected year-round.

Etymology.—From Malagasy for "round end" (borivody), in reference to rounded posterior margin of the anal division of the larva.

Eupaulianina botsimpatsy Sam, new species (Fig. 22)

Species H Stuckenberg 1958: 148-149.

Diagnosis.—Larva: Anterior four intercalary segments bisected completely, remaining intercalary segment slightly narrowed at midline. Chaetotaxy characterized by minute dark, pointed tubercles.

Description.— Larva (Fig. 22): Measurements, instar IV (n = 13): Total length 4.9mm (3.3-6.3), cranial width 0.49mm (0.44-0.54), basal antennal segment 0.16mm (0.12-0.17), apical antennal segment 0.2mm (0.17-0.22). Cranial sclerites dark brown with black margin posteriorly; ecdysial line with short stem line; frontoclypeal apotome extend nearly to posterior cranial margin. Anterior four intercalary segments bisected completely, remaining

intercalary segment slightly narrowed at midline. Coloration: Body uniformly brownish yellow. Raised dorsal plates pale brown, lighter then cranial sclerites. Lateral lobes with prolegs visible dorsally. Prolegs of cephalothorax tapered, narrowing apically, blunt apically; prolegs of anal division subcylindrical, blunt apically; remaining prolegs large, conical, tapered apically, rounded apically. Chaetotaxy: AL composed of three piceous, small, conical tubercles in vertical row, with upper tubercle twice as far from middle tubercle then basal tubercle; AD consists of 2-3 conical, piceous tubercles, slightly larger then AL; LP yellowish-brown, stout, conical; M present as subrectangular, smooth, light brown plate with two piceous, small, conical tubercles; AM present as subrectangular, smooth, light brown plate with three piceous, conical tubercles, larger then the tubercles on M, though still very small; PM present as subrectangular, smooth, light brown plate with two piceous, small, conical tubercles; PP present as small, piceous, conical tubercle; R present as two subrectagular plates, similar in size and shape to AM and PM, respectively each bearing two small, piceous, conical tubercles; S present as single subrectangular smooth, light brown plate bearing two small, piceous, conical tubercles; T absent. Clear division between last abdominal segment and anal segment lacking; Prolegs with large yellow spines and dense apical patch of long, yellow setae.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Fianarantsoa*: Ambalavao, Vakoana, 22 January 1958, coll. B. Stuckenberg. **Other Material examined**.—MADAGASCAR: *Toamasina*: Maroantsetra, Mangabe 30 Marc 1958. *Fianarantsoa*:

Ambalavao, Kimoro R., E. slopes of Ambaravarandanitra Mt. 19 January 1958. All specimens collected by B. Stuckenberg.

Distribution.—*Eupaulianina botsimpatsy* displays a wide range, being found in the northeastern forest of Maroantsetra and in the central and southern forests in Anstirabe and Andringitra. It is sympatric with *P. ingens*, *P. lehibe*, *E. alexanderi*, *E. pamela*, *E. rivalis*, *E. silva*, *E. telofantsy*, *E. korontsilo*, and *E. borivody*.

Bionomics.—Because collection has been so limited and only extends to larvae, it is difficult to draw conclusions regarding the species' behavior. Larvae were collected from January to March, which is normal for most larvae.

Etymology.—From Malagasy for "spines just beginning to grow" (botsimpatsy), in reference to the small size of the tubercles located on the dorsum of larva.

Eupaulianina korontantsilo Sam and Courtney, new species (Figs. 18, 33, 125-128)

Diagnosis.—Small blephariceridid. Larva: Chaetotaxy consists of conical tubercles located on plates, with random number of smaller tubercles less then ¼ size of prominent tubercles scattered across plates. Pupa: Papillae well developed, roughly ovoid, with rough corrugated margin; Single large lamellae with lobe along posterior medial edge.

Description.— Larva (Fig. 18): Measurements, instar IV (n = 20): Total length 4.1mm (3.3-5.4), cranial width 0.5mm (0.4-0.5), first antennal segment 0.11mm (0.08-0.13), second antennal segment 0.15mm (0.11-0.17). Cranial sclerites light brown with black margin posteriorly; ecdysial line without stem line; frontoclypeal apotome extends to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Prolegs of cephalothorax subcylindrical, blunt apically;

prolegs of anal division subcylindrical, narrow, tapered apically, directed posteriorly; remaining prolegs large, conical, tapered apically with blunt ends. Division between last abdominal segment and anal segment only slightly defined. Posterior margin of anal division roundly swollen. Coloration: uniformly light brown; sclerotized plated darker, tubercles dark piceous. Lateral lobes with prolegs visible dorsally, pale. Chaetotaxy: AD two sharply pointed conical tubercles; AL composed of several conical tubercles in a vertical line; M composed of a oval-shaped plate with slight anterior curve with two prominent conical tubercles mediolaterally, 3-4 smaller tubercles less then ¼ size of prominent tubercles scattered across plate with one on lateral sides of each large tubercles ; LP with large conical tubercles, piceous at tip, light brown at base, additional piceous tubercle located on ventral base of larger tubercle, very small conical tubercle located on dorsal base of larger tubercle; AM composed of a oval-shaped plate with 3 prominent rounded tubercles placed equidistant from the other across plate, middle prominent tubercle slightly anterior of lateral prominent tubercles, lateral prominent tubercles with smaller conical tubercles less then ¼ size of larger tubercles on either side, additional smaller conical tubercle sometimes present close to the middle tubercle; PM composed of two prominent conical tubercles placed toward lateral ends of oval-shaped plate, 4 conical tubercles less then ¼ the size of prominent tubercles placed evenly across plate, one on each lateral side of prominent tubercles, two placed evenly between the prominent tubercles; PP composed of two conical tubercles; R composed of single large trapezoidal plate, pattern of tubercles similar to AM and PM combined; S composed of small oval-shaped plate, two prominent conical tubercles laterally with single small tubercle placed medially; T present as two to four single pale tubercles with piceous tip. Lateral prolegs dense apical patch of long, yellow setae.

Pupa (Figs 33, 125-128): Measurements, undetermined: (N=1): total length 4.25mm, total width 2.25mm. Cuticle dark brown. Body bullet-shaped, rounding laterally at alar sclerites; Third abdominal segment swollen at lateral ends. Anal segment rounded. Papillae present, uniformly dispersed across cephalic sclerite, branchial tergites, metatergite, abdominal tergites, and anal tergite; absent on lateral section of alar sclerite; absent in two small half-moon patches immediately behind inner edge of respiratory organs. Papillae well developed, glabrous; ovoid; roughly corrugated along margin. Cuticle between papillae smooth, uniform in pigmentation. Respiratory lamellae dark brown, similar in shape to Species O. Respiratory organs elongated transversely. Respiratory organ with lamellae appear to be merged into one distinct lamella; a straight line posterior border, lateral end pointed, rounding anteriorly to a blunt edge along interior end, curving around to form one small lobe at the posterior medial edge. Single division starts where the straight edge and rounded edge meet along posterior margin, continuing anteriorly 2/3 across the organ.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Fianarantsoa*: Ambalavao, nr. Antanifotsy, Jomando R, 10 January 1958, coll. B. Stuckenberg. **Other Material examined**.—MADAGASCAR: *Fianarantsoa*: Ambalavao, nr. Antanifotsy, Jomando R. 10 January 1958 [L,P]; Ambalavao, Antanifotsy, Kimoro R. on eastern slopes of Ambaravarandanitra Mt., 19 January 1958 [L]; Ambalavao, Antanifotsy, Vakona R., 22 January 1958 [L]; Ambalavao, nr. Antanifotsy, Antsifotra R., 23 January 1958; W. Ranomafana, 11 September 1993; *Toamasina*: Manjakandriana, Ambatobaona, 1 April 1958. Specimens from Ranomafana collected by F. F. Stahrmuhler. All other specimens collected by B. Stuckenberg.

Distribution.—*E. korontantsilo* seems to be limited to the central and southern part of the eastern rainforest across a wide range of elevations (near sea level to 5000M). It is sympatric with *P. ingens*, *P. umbra*, *P. lehibe*, *E. alexanderi*, *E. pamela*, *E. silva*, *E. telofantsy*, *E. botsimpatsy*, and *E. borivody*.

Bionomics.—larvae and pupae collected from January to April. Unable to determine whether there is a difference in phenological patterns due to the different levels of altitude and the time difference between collections.

Etymology.—From Malagasy for "random spine" (korontantsilo), in reference to the seemingly random placement of small tubercles on the dorsal plates of the larval.

Eupaulianinan marangitsilo Sam, new species (Figs. 20, 129-132)

Diagnosis.—Large blephariceridid. Larva: AM with three large spines tipped with dark, pointed sensillum directed posteriorly; LP spines directed posteriorly; AD with three stout tubercles. Pupa: Papillae ovoid, smooth on top, margin with series of pointed structures. Respiratory organ broadly kidney-shaped, structure appears to consist of three lamellae merged into one structure.

Description.—Larva (Figs. 20, 129): Measurements, instar IV (N=12): Total length 6.16mm (5.7-6.7), cranial width 0.6mm (0.54-0.62), basal antennal segment 0.16mm (0.15-0.17), apical antennal segment 0.28mm (0.26-0.33). Cranial sclerites castaneus with black margin posteriorly; ecdysial line with little to no stem line; frontoclypeal apotome extends to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Prolegs of cephalothorax subcylindrical, blunt apically; prolegs of anal division subcylindrical, narrow, tapered apically, directed posteriorly;

remaining prolegs large, conical, tapered apically with blunt ends. Division between last abdominal segment and anal segment only slightly defined. Posterior margin of anal division round. Coloration: Uniformly light brown; sclerotized plated darker, tubercles dark piceous. Lateral lobes with prolegs directed ventrally, light brown. Chaetotaxy: AD with three sharply pointed conical tubercles tipped with pointed dark sensillum, directed upward; central tubercle largest, outer tubercle 2/3 height of central tubercle, inner tubercle 1/3 height of central tubercle. AL composed of three conical tubercles in a vertical line; M composed of a oval-shaped plate with slight anterior curve with two prominent conical tubercles on lateral ends; LP with large sharp tubercle, placed on light brown raised sclerite base; large tubercle curved posteriorly, with sharp small extension located ½ along tubercle; additional piceous conical tubercles scattered across sclerotized base; AM with three large spines tipped with dark, pointed sensillum directed posteriorly, two small tubercles placed directly next to lateral spines, along plate margin; PM composed of a oval-shaped plate with two small conical tubercles placed at lateral ends; tiny central conical tubercle, ½ size of lateral tubercles; PP composed of single small conical tubercles; R composed of single large trapezoidal plate, two anterior tubercles large, sharp, curved posteriorly with stout tubercle arising out of base of larger tubercle; large stout tubercles placed at posterior corners; single small conical tubercles placed along median line between larger anterior tubercles; one large tubercle centrally located; S composed of small oval-shaped plate, two prominent conical tubercles laterally; T positioned posteriorly; plate pale; two single tubercles. Lateral prolegs scattered with small sharp tubercles with a dense apical patch of long, yellow setae.

Pupa (Figs. 130-132): Description based on structures within mature 4th stage larva analyzed by SEM. Undetermined (n = 1). Papillae ovoid, smooth on top, margin with series

of pointed structures. Respiratory organ broadly kidney-shaped. Structure appears to consist of three lamellae merged into one structure, distinction between lamellae poorly defined. Organ evenly divided into three segments. Division between lamella 1 and 2 begins at apex of basal curve of organ, continuing anteriorly. Division between lamella 2 and 3 also begins at apex of basal curve of organ, continuing horizontally toward midline.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Fianrantsoa*: Ambalavao, Antanifotsy, Kimoro R. on eastern slopes of Ambaravarandanitra Mt., 19 January 1958, coll. B. Stuckenberg.

Distribution.—Collection limited to south-eastern Madagascar within the Andringitra Range. It is sympatric with *P. ingens*, *P. lehibe*, *E. alexanderi*, *E. rivalis*, and *E. tsilobe*.

Bionomics.–Larvae only collected at single location in January, therefore, difficult to determine any patterns.

Etymology.—From Malagasy for “largest middle spine” (marangitsilo), in reference to the fact that the middle spine is largest on the AM plates of the medial segments of the larva.

Eupaulianina ratsilo Sam, new species (Figs. 23)

Diagnosis.—Larva: AM with large sharp tubercle, curved posteriorly placed laterally, stout tubercle arising out of base of larger tubercle; 2-3 minute conical tubercles in horizontal line between larger tubercles.

Description.— Larva: Measurements, instar III (n = 3): Total length 2.81mm (2.3-3.4), cranial width 0.33mm, antennal segments 0.248mm (0.24-0.27); instar IV (n = 7): Total length 4.9mm (3.5-6.1), cranial width 0.52mm (0.43-0.56), basal antennal segment 0.15mm (0.13-0.18), apical antennal segment 0.26mm (0.24-0.27). Cranial sclerites castaneus with black margin posteriorly; ecdysial line with little to no stem line; frontoclypeal apotome extends to posterior cranial margin. First intercalary segment bisected completely, remaining intercalary segments narrowed at midline. Prolegs of cephalothorax subcylindrical, blunt apically; prolegs of anal division subcylindrical, narrow, tapered apically, directed posteriorly; remaining prolegs large, conical, tapered apically with blunt ends. Division between last abdominal segment and anal segment only slightly defined. Posterior margin of anal division round with sclerite T directed posteriorly. Coloration: Uniformly light brown; sclerotized plated darker, tubercles dark piceous. Lateral lobes with prolegs directed ventrally, light brown. Chaetotaxy: AD two sharply pointed conical tubercles, innermost tubercles curved medioposteriorly; AL composed of three conical tubercles in a vertical line; M composed of a oval-shaped plate with slight anterior curve with two prominent conical tubercles on lateral ends; LP with large sharp tubercle, placed on light brown raised sclerite base; large tubercle curved posteriorly, with sharp small extension located ½ along tubercle; additional piceous conical tubercles scattered across sclerotized base; AM with large sharp tubercle, curved posteriorly placed laterally, stout tubercle arising out of base of larger tubercle; 2-3 minute conical tubercles in horizontal line between larger tubercles; PM composed of a oval-shaped plate with two small conical tubercles placed at lateral ends; tiny central conical tubercle, ½ size of lateral tubercles; PP composed of single small conical tubercles; R composed of single large trapezoidal plate, two anterior tubercles large, sharp,

curved posteriorly with stout tubercle arising out of base of larger tubercle; large stout tubercles placed at posterior corners; single small conical tubercles placed along median line between larger anterior tubercles; S composed of small oval-shaped plate, two prominent conical tubercles laterally with single minute tubercle placed medially; T positioned posteriorly; plate pale; two single tubercles. Lateral prolegs scattered with small sharp tubercles with a dense apical patch of long, yellow setae.

Pupa: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Type Material.—Holotype [4th stage larva]: MADAGASCAR: *Toamasina*: Maroantsetra, Mahalevona, Ambohitsitondroina Mt., 24 March 1958, coll. B. Stuckenberg.

Distribution.—Collection limited to north eastern Madagascar on the Masoala peninsula. It is sympatric with *E. marobotsin*.

Bionomics.—Larvae only collected at single location in March, therefore, difficult to determine any patterns.

Etymology.—From Malagasy for "someone with spines" (ratsilo), in reference to the stout double spines locates on the lateral margins of the AM plates located on the dorsum of the larva.

Eupaulianina robinsoni (Alexander) (Figures absent)

Syn: *Paulianina robinsoni* Alexander 1956

Eupaulianina robsinsoni Alexander 1956: 51, 75-81. Stuckenberg 1958: 156-157.

Entire description based on Stuckenberg (1958) and Alexander (1956), from a single type specimen, adult female, collected on route between Anosibe and Toamasina in February 1955.

Diagnosis.—Medium blephariceridid. R_3 passes very close to R_{1+2}.

Description.—Larvae: unknown

Pupa: Unknown.

Adult male: Unknown.

Adult female: Size: medium. Measurements (n = 1): Wing length 5.63mm, width 2.0mm.

Head: Labrum equal to about 2.3 of basal flagellar segment, uniform in width, apex quite broadly rounded, tip acute. Palpi with 5 palpomeres, distal 4 segment proportions 15:17:10:12.5. Second segment moderately swollen. Antennal segments covered with fine, inconspicuous microtrichia; apical segment narrower and distinctly longer then penultimate segment.

Thorax: Stigma elongate, ending close to for of R_3 and R_4. Spur 0.66 length of r-m.

Coloration: Unknown

Terminalia: Eighth sternite with elongate reniform window at base of median fold. Oviscapt moderately broad, apical lobes narrow, slender, widely separate by a rectangular excision.

Type Material.—Holotype [adult female, dissected]; MADAGASCAR. Toamasina: km 57, route Anosibe – "Tanarive"/Toamasina, February 1955, coll. A. Robinson.

Notes.—Stuckenberg (1958) notes that while specimen was dissected, one front leg, one middle leg, one antennae and a maxillary palp are missing. Stuckenberg also noted that the most closely related species seems to be *E. pamela*, based on the spermathecae.

Species K (Fig. 35)

Description.—Pupa: Measurements, male (n = 2): Total length 4.2mm, width 2.3mm. Cuticle dark brown. Body roughly ovoid, alar sclerites severely constricted; Anal segment round. Papillae present, uniformly dispersed across cephalic sclerite, branchial tergites, metatergite, abdominal tergites, and anal tergite; absent on lateral sections of alar sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory lamellae dark brown. Respiratory organs elongated transversely. Five lamellae present as swellings, raised centrally to give the appearance of a mound in the middle of each; lamella 1 large, rounded along outer margin, with straight edge along posterior margin; lamellae 2 and 3 ovoid, curved towards each other posteriorly; lamella 2 extended into lobe that curves posteriolaterally around to meet lamella 1; lamella 6 ovoid.

Larva: Unknown.

Adult male: Unknown.

Adult female: Unknown.

Material examined.—MADAGASCAR: Toamasina, Maroantsetra, Ambodivohangy, 16-20 March 1958 collected by B. Stuckenberg.

Distribution.—Restricted to the northeastern peninsula within the forested region of Maraontsetra.

Species L (Fig. 36)

Description.—Pupa: Measurements, undetermined (n = 1): Total length 4.0mm, width 2.3mm. Cuticle light brown. Body ovoid with posterior margin, including anal segment

slightly truncated. Papillae present, uniformly dispersed across cephalic sclerite, branchial tergites, metatergite, abdominal tergites, and anal tergite; absent on lateral sections of alar sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory lamellae castaneus. Respiratory organs elongate transversely, kidney-shaped. Lamellae appear merged into three separate lobes, the first lamella large with a straight edge along posterior margin, sharply rounded at outer margin, and rounded along anterior margin, bluntly meeting lamella 2; lamellae 2 triangular in shape, cornered with a rounded anteriolateral margin; lamella 3 formed poster rounded lobe, with a truncated anterior margin placed next to lamella 2.

Material examined.—MADAGASCAR: *Antsiranana:* FM 207, Isaka, 35 km North of Fort Dauphine, 9 September 1958. Collected by F. F. Stahrmuhler.

Distribution.— Single specimen collected, apparently limited to the far southern region of Madagascar.

Species M (Fig. 37)

Description.—Pupa: Measurements, Undetermined: (N=1): Total length 4.4mm, total width 2.1mm. Cuticle pale brown. Body narrowly ovoid. Anal segment rounded. Papillae present, uniformly dispersed across cephalic sclerite, metatergite, abdominal tergites, and anal tergite; dispersal lighter immediate behind respiratory organs on branchial sclerite; absent on lateral margins of alar sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory lamellae dark brown, half moon shape. 5 lamellae wide bands, positioned uprightly, with evident space between. Lamella 1 rectangular with point towards lateral margin of pupa; lamellae 2-4 rectangular, upright, with

flattened tops, placed along perpendicular line to within respiratory organ; lamella 6 rounded, upright.

Material examined.—MADAGASCAR: *Ambalavao:* Jomando R. nr Antnifotsy, 10 January 1958. Collected by B. Stuckenberg.

Distribution.—Single specimen restricted to the Jomando River, Andringitra NP region.

Species N (Fig. 38)

Description.—Pupa: Measurements, Undetermined: (n = 1): Total length 4.2mm, total width 2.25mm. Cuticle light brown. Body ovoid. Anal segment broadly rounded. Papillae present, uniformly dispersed across cephalic sclerite, metatergite, abdominal tergites, and anal tergite; absent on lateral margins of alar sclerite; distinct line without papillae starting just inside the central posterior margin of the respiratory organ, moving posteriolaterally towards the tip of the division between alar and branchial sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory lamellae chestnut-brown, very similar in structure to Species K. Respiratory organs elongated transversely. Five lamellae present as swellings, raised centrally to give the appearance of a mound in the middle of each; lamella 1 large, rounded along outer margin, with inwardly-curved edge along posterior margin, lamella constricted at interior margin where it meets lamella 2; lamellae 2 and 3 ovoid, curved towards each other posteriorly; lamella 2 extended into lobe that curves posteriolaterally around to meet lamella 1; lamella 6 ovoid.

Material examined.—MADAGASCAR: *Antsiranana:* FM 213, Androkabe, 40 km north of Fort Dauphine, 10 September 1958. Collected by F. F. Stahrmuhler.

Distribution.—Single specimen restricted to southeastern mountainous region north of Fort Dauphine.

Species O (Fig. 39)

Description.—Pupa: Measurements, Undetermined: (n = 1): total length 4.0mm, total width 2.25mm. Cuticle golden brown. Body ovoid. Anal division broadly rounded. Papillae present, uniformly dispersed across cephalic sclerite, metatergite, abdominal tergites, and anal tergite; absent on lateral margins of alar sclerite; distinct line without papillae starting just inside the central posterior margin of the respiratory organ, moving posteriolaterally towards the tip of the division between alar and branchial sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory organs golden brown. Respiratory organs elongated transversely; broadly kidney-shaped, similar in shape to those of *E. korontantsilo*. Lamellae appear to be merged into one distinct lamellae; a straight line posterior border, lateral end pointed, rounding anteriorly to a blunt edge along interior end, curving around to form one large rounded lobe at the posterior medial edge. Single incomplete division starts where the straight edge and rounded edge meet along posterior margin, continuing anteriorly 2/3 across the organ.

Material examined.—MADAGASCAR: *Fianarantsoa:* FM 136, Isaka, 20 km north of Ranomafana, 7 August 1958. Collected by F. F. Stahrmuhler.

Distribution.—Single specimen restricted to southeastern rainforest near to Ranomafana.

Species P (Fig. 40)

Description.—Pupa: Measurements, Undetermined: (n = 2): total length 3.6mm (2.6-3.9), total width 1.47mm (1.45-1.5). Cuticle pale. Body narrowly ovoid. Anal division rounded. Papillae present, uniformly dispersed across cephalic sclerite, branchial tergites, metatergite, abdominal tergites, and anal tergite; absent on lateral part of alar sclerite. Papillae well developed, ovoid. Cuticle between papillae uniform in pigmentation. Respiratory lamellae light brown. 4 distinct lamellae, present as swellings with limited space between. Lamella 1 oblong, sharply rounded at outer lateral margin, roundly curved along both anterior and posterior margins; lamellae 2 and 3 both ovoid, angled slightly laterally; lamellae 4 J-shaped, round at interior margin, curving around the base of both lamellae 2 and 3 to reach posterior margin of lamella 1.

Material examined.—MADAGASCAR: *Antsiranana:* Rousettes Forest Station nr. Montage d.Ambre, 23 November – 4 December 1958. Collected by B. Stuckenberg.

Distribution.— Two specimens restricted to the far northern forest ranges near Montagne d'Ambre.

Taxonomic Notes

Paulianina hova was described from a single female specimen collected on Mt. Tsaratanana in northern Madagascar. *Paulianina ingens* was described based on adult male specimens also collected at Mt. Tsaratanana as well as four other locations. Because adult females are unrecorded for *P. ingens* and the only known specimen of *P. hova* is sympatric with male *P. ingens*, it is possible that that these two species represent different sexes of the same species. The major characteristic separating the two species is the relationship between the M_4 and Cu wing veins.

The mouthparts of larval *Paulianina* are uniquely derived in many aspects. The greatly enlarged maxillae are the most striking characters, however, as Zwick (1977) noted, the larvae display other mouthpart modifications. A loss of pectinate teeth on the lacinial pads and the simple labial structure could be modifications associated with the enlargement of the maxillae (Zwick 1977). Zwick (1977) also noted that the mandible does not act as a scraping organ as it does in other larval blephariceridsbut rather functions as one with the maxilla, moving the large brush-like structure across the surface, so that the maxilla itself acts as the scraper. In this respect, *Paulianina* larvae are behaving more like a "browser", preferentially consuming the upper layers of the periphytic canopy.

Scanning electron microscopy (SEM) provided additional detail regarding mouthpart structure. The maxillary palpi of *Edwardsina* possess 12 sensilla, whereas those of both *Paulianina* (Fig. 48) and *Eupaulianina* (Fig. 70) display 10, having lost the anterior C sensillum as well as an extra sensillum close to the F sensilla in *Edwardsina*. These extra sensilla in *Edwardsina* remain problematic, but could be homologous to one of many

unidentified maxillary sensilla in larval Deuterophlebiidae. Assuming a sister-group relationship with deuterophlebiids, 10 sensilla is the apomorphic state for the rest of the Blephariceridae. SEM also revealed that reticulation along the proximal edge of the palpus is autapomorphic in *Paulianina*. A similar type of reticulation occurs in *Horaia* Tonnoir (Apistomyiini), but lies on the opposite side of the palpus. Moving from more basal to more derived lineages, there appears to be a loss of clypeolabral sensilla. *Edwardsina* has eight prominent sensilla and two less prominent sensilla on either side. The trend continues in *Eupaulianina*, which has 6 prominent sensilla, and *Paulianina* with five to six, and distributed more vertically than in either *Edwardsina* or *Eupaulianina*. Outside of Edwardsininae, only *Philorus* Kellogg has four sensilla, while the remaining genera have two.

Larval chaetotaxal microsculpture provided key characters in species recognition. Both *E. rivalis* and *E. tandroka* have chaetotaxy that appears similar under the light microscope (Figs 13, 24), but was revealed by SEM to be unique (Figs. 83-84, 110-112), confirming their placement as separate species. However, their general chaetotaxy (plates extending across the entire abdominal segment, covered with closely placed raised microsculpture) suggests a possible sister-species relationship.

Eupaulianina vohimalama (Figs. 21, 113-115) displays characteristics of both genera. While the species lacks the large maxillary palpus that so clearly distinguishes members of *Paulianina*, its anal division is not clearly demarcated, appearing tri-lobed, a plesiomorphic trait in *Paulianina* and absent in all other species of *Eupaulianina*. It is also the only *Eupaulianina* larva that lacks tubercle-like structures across the dorsum, instead displaying simple flat plates in the AM and PM positions.

The aedeagal filaments of *E. fantsona* (Figs. 148, 149) enter the ventral parameres. This characteristic is shared by *E. alexanderi*, *E. silva* and *E. tsilobe* and represents a potential synapomorphy of the *E. silva* group. However, because *E. fantsona* is not included in the morphological analysis (because only the adult stage is available) or the molecular analysis, the placement of this species in this group is tentative.

Character Analysis

Alternative character states used in the morphological phylogenetic analysis include (plesiomorphic characters = 0; apomorphic characters = 1, 2, 3):

Larval characters

1. 3rd instar antennae	0. single segmented
	1. two segmented
2. Maxilla size	0. at least 1/5 size of head capsule
	1. at least ½ size if head capsule
3. Maxillary palpus	0. with 12 sensilla
	1. with 10 sensilla
4. C-sensilla of maxillary palpus	0. present
	1. absent
5. Maxillary palpal reticulations	0. absent
	1. present along proximal edge
	2. present on both sides of palp
6. Clypeus	0. with 8 sensilla
	1. with 5-6 sensilla

7. Cranial sclerite margin	0. straight
	1. produced posteriorly in median line as small lobe less then ½ length of head capsule
	2. produced posteriorly in median line as broad lobe ½ length of head capsule
8. Intercalary segments	0. not divided
	1. divided
9. Dorsal sclerotization	0. absent
	1. covers dorsum less then ¾ of space
	2. covers dorsum more then ¾ of space
10. Dorsal plates	0. absent
	1. present
11. AM plate	0. tubercles absent
	1. with 1-3 tubercles
	2. with > 3 tubercles
12. Dorsal chaetotaxy	0. absent
	1. consists of simple, small sensilla
	2. consists of stout conical sensilla
	3. consists of elongate conical sensilla
13. Anal division	0. appears tri-lobed, anal prolegs not clearly demarcated from anal division
	1. does not appear tri-lobed, prolegs clearly demarcated from anal division

14. Anal division	0. clearly demarcated from abdominal segments
	1. not clearly demarcated from abdominal segments
15. Posterior tubercles on T plate	0. absent
	1. present
16. AM plate sensilla	0. Absent
	1. 3 sensilla of equal length
	2. 3 sensilla of different length

Pupal characters

17. Papillae	0. lacking any extensions
	1. with elongate extensions
	2. with globose extensions
18. Papillar margin	0. scalloped or rough
	1. smooth
19. Papillar spines	0. absent
	1. present
20. Respiratory organ lamellae	0. clearly separate
	1. combined separate and lobed swelling
	2. present as swellings
21. Respiratory organ	0. with 10 lamellae
	1. with 6-9 lamellae
	2. with ≥ 5 lamellae

Adult characters

22. Aedeagal filaments	0. tubular
	1. lanceolate
23. Maxillary palpi	0. 5 segmented
	1. 4 segmented
24. Tibial spur formula	0. 1-0-1
	1. 1-1-1 (apomorphic).
25. Anal vein	0. complete
	1. incomplete
26. M_3 and M_4	0. connected at base
	1. not connected at base
27. Aedeagal filaments	0. separated from parameres
	1. aligned with parameres
28. Ventral parameres	0. present
	1. absent

Phylogenetic Results

Within Blephariceridae, both *Paulianina* and *Eupaulianina* are now supported as monophyletic; 28 characters and 15 taxa were evaluated through the morphological parsimony phylogenetic analysis; 28 characters and 12 taxa were evaluated through the morphological Bayesian phylogenetic analysis. Additional analyses using molecular characteristics from one nuclear gene and one mitochondrial gene were performed using Bayesian techniques.

Parsimony analysis of morphological data. (Fig. 150) The tree produced using bootstrap analysis had very low support (CI = 0.54, RI = 0.63). *Paulianina* is presented as a paraphyletic group. *Eupaulianina* has high support (Bootstrap = 100) as a monophyletic group, however, within the group itself, there was little resolution (Bootstrap = 58), except *E. vohimalama* was placed as the sister species to the remainder of *Eupaulianina*. Only the larval and pupal characteristics were used for *E. telofantsy*, *E. borivody*, *E. korontantsilo*, *E. marobotsin*, and *E. vohimalama*.

Because adult data for many species were incomplete, a second analysis was performed using only characters from the larvae and pupae. The resulting tree (Fig. 151) also had low support (RI = 0.78, CI = 0.65). *Paulianina* was again paraphyletic while *Eupaulianina* had high support (Bootstrap = 99). Relationships within *Eupaulianina* have almost no resolution (Bootstrap = 64), except *E. vohimalama* is again placed as sister to the remainder of *Eupaulianina*. *Eupaulianina rivalis* is also placed outside the large polytomy that includes most of *Eupaulianina* species, aligned next to *E. vohimalama* at the base of the clade (Bootstrap = 88).

Bayesian analysis of morphological data. (Fig. 152) Compared to the results of the parsimony analysis, the Bayesian analysis of morphological data provided some support for *Paulianina* and *Eupaulianina* as sister clades, though the support for *Paulianina* is insignificant, the posterior probability only at 0.68. While the support for *Eupaulianina* is much higher at 0.99, the relationships within *Eupaulianina* were again poorly resolved. This could be in part due to the fact that not all life stages were available, causing the larval stage to carry more weight during the analysis, and leaving gaps in the adult data set. Despite this, monophyly of the *E. silva* group was not well supported with a posterior probability of 0.69

and *E. rivalis* and *E. tandroka* were grouped together with a low posterior probability of 0.73. The remaining relationships within *Eupaulianina* remained unresolved.

Bayesian analysis of BZF2. (Fig. 153) For the nuclear BZF2 gene, 1232 bp were generated. After alignment adjustments, 1000bp were analyzed. While the nuclear gene supports *Paulianina* and *Eupaulianina* as two separate clades, it also does not provide high resolution of relationships within *Eupaulianina*. *Eupaulianina rivalis*, *E. trandroka* and *E. pamela* are grouped together, though as an unsupported polytomy within the clade.

Bayesian analysis of ND2. (Fig. 154) For the mitochondrial gene, ND2, 1303 bp were generated. After alignment adjustments, 1300bp were analyzed. The ND2 mitochondrial tree provides full support for *Paulianina* and *Eupaulianina* as separate clades, with each clade having a posterior probability of 1.0. The tree also provides much better resolution within *Eupaulianina* itself, with many nodes showing posterior probabilities > 0.7, However, the relationships between species are mostly fully supported. Again, as in the morphological tree, both the *E. silva* group and the *E. rivalis* + *E. tandroka* sister species are well supported (posterior probabilities of 0.72 and 1.0 respectively).

Bayesian analysis of the combined dataset. The analysis using both sets of molecular data (ND2 +BZF2) combined with the morphological matrix produced the tree with the overall highest resolution of relationships within each group (Fig. 155). The tree also provided strong support *Paulianina* and *Eupaulianina* as sister clades, with each clade having a posterior probability of 1.0, while providing good resolution within *Eupaulianina*. The relationships of *E. marobotsin*, *E alexanderi*, *E. pamela*, and *E. vohimalama* to the *E. silva* group and the *E. rivalis* grouping was consistent with the mitochondrial tree, indicating that the resolution of relationships is stronger as additional data sets are added to the analysis.

Phylogenetic Discussion

Monophyly of the Malagasy Edwardsininae is supported by the following synapomorphies: 1) reduction of the base of M_3 (Zwick 1977); 2) 4th stage larvae with two segmented antennae (Zwick 1977); and 3) fusion of tergite and sternite VIII into a complete annular sclerite in both sexes (Stuckenberg 1958). While the placement of *Paulianina* and *Eupaulianina* within the Edwardsininae is well-supported (Zwick 1977, Wood and Borkent 1989, Courtney 2003, Arens 1998), there has been much speculation regarding the monophyly of these taxa (Zwick 1977, Arens, 1998). Stuckenberg (1958) originally designated *Eupaulianina* as a subgenus because he was concerned that placing it separately as a genus would interfere with the monophyletic nature of the Malagasy group as a whole. Subsequent analyses by both Zwick (1977) and Arens (1998) determined that *Eupaulianina* was well supported as a monophyletic group, but both questioned the monophyly of *Paulianina*.

Paulianina shares many morphological characteristics with *Edwardsina*. This is supported by phylogenetic trees from the current analysis (Figs. 150-155), all of which place *Paulianina* as the sister to the remainder Malagasy blephariceridsas the genus shares the most characters with *Edwardsina*. Characters shared by both genera include: 1) lack of noticeable dorsal chaetotaxy (except in *P. lehibe*); 2) antennae bisegmented in 4th stage larvae. *Eupaulianina* larval antennae are bisegmented in 3rd instars. The most derived state, the larval antennae bisegmented at the 2nd larval stage is found throughout the Blephariceriae; 3) pupal respiratory lamellae clearly defined and separated; 4) larval clypeal setae numbering between 8-10; 5) complete intercalary divisions; 6) larval anal division appears to be tri-lobed; 7) pupal papillae lack spines; and 8) adult aedeagal filaments tubular.

These symplesiomorphs created some confusion when determining the monophyly of *Paulianina* as a whole. However, *Paulianina*'s plesimorphic characters consistently place it at the base of the Malagasy clade.

While both Stuckenberg (1958) and Zwick (1977) asserted that the unusual mouthpart structure of *Paulianina* larvae provided sound evidence for monophyly, Arens (1995, 1997, 1998) questioned this assertion based on his very thorough analysis of the group's unique pupal spiracular gill structure. Arens (1998) determined that indeed *P. ingens* and *P. umbra* were closely related; however, many derived similarities between the gill structure of *P. umbra* and members of *Eupaulianina* caused him to propose a new phylogeny, placing *Paulianina* as a paraphyletic group. While the pupal respiratory organ is a compelling character, the determination of paraphyly based on a single character ignores many other strong characters from other life stages. The monophyly of *Paulianina* is supported by several morphological synapomorphies: 1) larvae with greatly enlarged maxillae; 2) reticulations along the proximal edge of the maxillary palpus; and 3) larval antennae two segmented starting in 3rd instar. The current analysis based on both morphological and molecular characters provides strong support that both groups are indeed monophyletic.

The monophyly of *Eupaulianina* is supported by the following morphological synapomorphies: 1) larvae with six clypeal labral sensilla; 2) larval antennae two segmented starting in 4th instar; 3) pupal papillae with elongate extensions; and 4) pupal respiratory lamellae reduced to large swellings (except in *E. tsilobe*). Within *Eupaulianina* it appears that elongation of the dorsal spines is a derived state, supported by the placement of both *E. vohimalama* (lacking any spines) and *E. alexanderi* (spines are stout, shortened) as the sister species to the rest of *Eupaulianina*. *Eupaulianina rivalis*, *E. tandroka*, *E. pamela* and *E.*

marobotsin all display short, stout spines as well. The *E. silva* group appears to be the most derived, with all constituent species displaying large, elongate spines along their dorsal plates. (See Appendix, Figs. 1-2). The papillae of pupal *Eupaulianina* are unusual in many respects, especially the presence of lobed extensions in many species. While it is clear that pupal microsculptural features are synapomorphic, and were expected to play a significant role in the determination of relationships within the genus, their presence in the matrix did not improve the support values of the tree. However their removal caused the complete collapse of the *Eupaulianina* clade during bootstrap analysis, indicating they do play some role in the phylogenetic structure of the group.

Although pupal microsculpture was important in species identification, the characters associated with microsculpture provided little phylogenetic signal. For example, the unusual projections that extend from the papillae of many *Eupaulianina* appear to have originated independently in several members of the group. Interpreting the distribution of this and other microsculptural characters is confounded by our lack of understanding of the function of these structures, be it some hydraulic purpose or something else entirely.

An important adult synapomorphy pertains to the male aedeagal structure. The plesiomorphic condition includes filaments that are elongate and tubular. In both *E. pamela* and *E. rivalis* these filaments are lanceolate, a character state that links these two species. Because the adult stage of *E. tandroka*, the apparent sister species to *E. rivalis*, is unknown, it cannot be stated whether this aedeagal character represents a synapomorphy of these three species. Another derived character that helps define members of the *E. silva* group is the relationship between the aedeagal filaments and ventral parameres. The aedeagal filaments of *E. fantsona* (Figs. 148, 149) enter the ventral parameres. This characteristic is shared by *E.*

alexanderi, *E. silva* and *E. tsilobe* and represents a potential synapomorphy of the *E. silva* group. However, because *E. fantsona* is not included in the morphological analysis (because only the adult stage is available) or the molecular analysis, the placement of this species in this group is tentative.

Both morphological and molecular characters were considered when deciding to raise the two subgenera *Paulianina* s. s. and *Eupaulianina* to generic status. Each group has several morphological synapomorphies to support them as separate genera. Several alternate phylogenetic analyses, incorporating both morphological and molecular characters, were performed to thoroughly examine the relationship between the two groups to determine if each group would be raised to generic status.

The parsimony analyses did not provide strong support for raising the two subgenera to separate genera (Figs. 150-151). However, the dataset lacked a large number of adult characters that, if included, could possibly provide much stronger resolution within the tree. The Bayesian analysis of morphological data, which includes fewer taxa but all life stages, provided stronger support values for a monophyletic *Paulianina* (Fig. 152). This suggests that the addition of *P. lehibe* (without adult scored characters) in the parsimony analyses may contribute to the paraphyly of *Paulianina* (Fig. 150). The analysis using only larval and pupal characters did not provide any additional resolution (Fig. 151). Using only the subset of 18 characters failed to increase the resolution within either clade (See Appendix, Figs. 4-5). Overall, the parsimony analyses suggest that adult characters could indeed play an important role in resolving the phylogentic relationships of the group. Additional analyses incorporating all life stages of known species are needed to test these hypotheses and provide further resolution of relationships.

Additionally, none of the parsimony analyses provided sound resolution of the relationships within each genera. *Eupaulianina vohimalama* is unique within *Eupaulianina*, displaying several plesiomorphic characteristics (lack of chaetotaxy, the anal prolegs not clearly demarcated from the anal division) seen in *Paulianina*. All parsimony analyses supported the placement of this species as the sister to the remainder of the *Eupaulianina* clade (150-151). *Eupaulianina rivalis* is also placed basally in the larvae + pupae analysis, however the remaining relationships are undetermined in both parsimony analyses.

Because fresh specimens were required for molecular studies, several species could not be included in the molecular and combined analyses. Many of these species were collected only during Stuckenberg's 1958 expedition and were therefore unsuitable for molecular analysis. Despite this, molecular studies provided new insights into relationships both between and within the two genera.

The analysis using only the nuclear gene did not provide strong resolution (Fig. 153). This is not surprising for a nuclear gene, as they are much more conserved and generally thought to be more useful when used for determining higher-level phylogenetic relationships (Lin and Danforth 2004). However, the current study was one of the first to use BZF2 for blephariceridae, and has provided sound initial data for the group, specifically regarding the basic structure of each clade as well as initial support for raising *Paulianina s.s.* and *Eupaulianina* as separate genera. However, this is the only molecular analysis that does not place *E. vohimalama* as the sister to the rest of the *Eupaulianina* clade, pairing it instead with *E. alexanderi*, with both these species nested within the remaining members of the *E. silva* group. Note that this grouping has no support which should be considered when analyzing relationships.

The mitochondrial data (Fig. 154) placed both *Paulianina* and *Eupaulianina* as sister clades with strong posterior probabilities of 1.0 for each. This gene also provided much better resolution of the relationships within *Eupaulianina*. *Eupaulianina vohimalama* was placed in a basal position within the *Eupaulianina* clade. The relationships between members of the *E. silva* group are fully resolved, with monophyly of several groups strongly supported. All of the following relationships: *E. marobotsin* ((*E. pamela*) *E. rivalis* + *E. tandroka*)), were also supported with posterior probabilities of 1.0. Some of these *Eupaulianina* relationships appear consistent from both the morphological and molecular analyses.

This study was the first to use both morphological and molecular characters in a combined analysis of relationships among Malagasy Edwardsininae. The resulting tree (Fig. 155) displayed the highest level of support in regards to raising *Paulianina s.s.* and *Eupaulianina* to separate genera. Strong resolution of relationships within *Eupaulianina* is similar to that of the mitochondrial analysis, supporting the specific relationships discussed above throughout the clade. *Eupaulianina vohimalama* is again placed as the sister species to the rest of the *Eupaulianina* species. *Eupaulianina alexanderi*, however has been placed outside of the *E. silva* group as the sister species for the remainder of *Eupaulianina*. A clade supporting the relationship: *E. marobotsin* ((*E. pamela*) *E. rivalis* + *E. tandroka*)) is again supported with a posterior probability of 1.0, while the remaining members of the *E. silva* group (*E. tsilobe* (*E. silva* + *E. telofantsy*)) is supported with a posterior probability of 0.92.

Morphological and molecular data support recognition of all new species. Also, the urgent need to document the diversity of the family in face of ongoing habitat destruction provides an impetus for providing names. Among *Eupaulianina*, adults have been associated with both larvae and pupae in all species except *E. fantsona*. The latter species appears to be

endemic to a single location where larval and pupal collections suggest the presence of a second species, *E. vohimalama*. Although possible that some of these larval and pupal collections represent *E. fantsona*, this seems unlikely because *E. vohimalama* displays a unique combination of plesiomorphic characterstics and known life stages of *E. fantsona* possess morphological characteristics that tentatively place it in the relatively derived *E. silva*-group.

Overall, morphological characters alone provide little information regarding the relationships between the two genera or within each genus. However, the molecular data clearly provide stronger resolution regarding relationships at both the species and generic level. It is clear that although the additional molecular analysis provides a sound basis for raising the two subgenera to generic status, more collection and further molecular analyses are needed to address the remaining questions regarding the relationships within each clade. This includes species lacking one or more life stage. In order to resolve these questions, fresh specimens must be made available. Whether or not this will be possible in the future remains to be seen.

Biogeographical Discussion

Madagascar was once part of the large landmass known as Gondwana, sandwiched mainly between between what is now Africa and India over 170mya. When Gondwana began to break up, Madagascar first began to split from the African mainland approximately 160mya (Yoder and Nowak 2006), but continued to have a close association with the landmass composed of India, Antarctica, and Australia until India and Madagascar completely separated sometime around 130mya (Briggs 2003). Madagascar stayed joined

with India until approximately 100mya, though there is some debate regarding the exact timing of this event, though the break-up took a long time, covering a time period of up to 30 million years (Seward et al. 2004). India continued its northward path until it collided with Asia between 55-50mya (Beck et al. 1995; Zhu et al. 2005).

The isolation of Madagascar for 65 million years has facilitated speciation in many taxa, including net-winged midges (Goodman and Benstead 2003). This has resulted in a higher collected diversity than expected, with many additional species almost certainly residing in uncollected areas. Madagascar has remained a biogeographical puzzle for many who study the island's diversity. While located only 400 km from the African mainland, the biota of Madagascar is most often closely related to that of Africa (Yoder and Nowak 2006) as expected, but continues to show high affinities with the Indian plateau (Warren et al. 2010). Several theories have been proposed for this Asian influence, especially as much of the diversity seems to have arrived on the island within the last 87 million years, after break-up of Gondwana (Warren et al. 2010). Briggs (2003) hypothesized that during the separation of the continents the tip of India stayed in close contact with Madagascar longer then originally thought. Warren et al. (2010) proposed that the rise and fall of sea levels exposed several additional islands that would have allowed for easier dispersal between Asia and Madagascar.

However, Edwardsininae presents a unique biogeographical puzzle because it shows no affinities with other members of the family found in either Africa or India. The group follows the classic Gondwanan distribution, being collected on the continents of South America, Australia, and Madagascar. However, these flies have never been found on the African mainland and are not present in India. The question isn't how the group dispersed

across Gondwana, but why they are lacking from the African mainland, which was on a direct route between Australia/Madagascar and South America when the continents were joined 200 million years ago. Because the group is so tied to its natal habitat their movement is usually attributed to vicariance events rather then long-distance dispersal (Edwards 1929). This question remains unanswered, though perhaps as more species are sequenced, more information will be revealed.

There are few geographical trends within the group on the island of Madagascar itself. *Paulianina* and *Eupaulianina* are dispersed all across the island in forested areas (Table 2). There is some evidence of altitudinal separation of blephariceridspecies on the island (See Table 3). Ten species were collected at or below 1000 meters elevation, with both *Paulianina umbra* and *Eupaulianina alexanderi* being collected as low as 300 meters elevation. Eleven species were collected at or higher than 1500 meters in elevation. Based on known collection records, there appears to be a species void between 1000 and 1500 meters. Thus, there seems to be two major distributional patterns, a low-elevation group and a high elevation group. However, despite the obvious distinction between the two elevation groups, we found no evidence of endemism based on elevation, with only two species restricted to the two elevation ranges. *Eupaulianina rivalis* and *E. vohimalama* are specific to the low elevations, while only *E. telofantsy* and *E. korontantsilo* appear to be restricted to high elevations. *Paulianina ingens* has been collected mainly at higher elevations, although Stuckenberg (1958) collected one specimen at approximately 700 meters. Such trends could also be merely a collecting artifact. It is also important to note that altitudinal data were not available for all species, based on collection records from six decades ago or more. When

possible, locations were matched with altitudinal data, however that was limited to well known areas such as national parks. In more obscure areas such data is unavailable.

Stream conditions appear to affect the distribution of species to a larger degree than elevation (Table 4). Three species were located in only one habitat: *E. marobotsin* was collected only in the splash zone, whereas both Sp. J and *E. korontantsilo* were collected exclusively in the madicolous habitat. Three species, *Paulianina umbra*, *Eupaulianina tsilobe*, and *E. tandroka*, were restricted to the splash/madicolous zones. Only one species, *Eupaulianina rivalis*, was collected in both the madicolous zone and the deeper riffles of small streams. *Paulianina ingens* spanned a greater range of habitat, being collected in the madicolous zones as well as both small and large streams. Only three species, *Eupaulianina alexanderi*, *Eupaulianina pamela*, and *Eupaulianina silva*, were found in all habitats. Again, for the same reasons discussed above, data were not available for all species, and must be considered when looking at trends between habitat types.

There was some question regarding the presence of these flies in the small highland forest fragment found within the Special Reserve Ambohitantely. As the adults rarely leave their natal habitat, it can be assumed that there must have been a pathway between the eastern rainforests and the fragments found in the highlands, which when regarding the current topography of the area seems unlikely. However, looking at the history of the island reveals the answer. The use of the word forest fragment indicates that the highlands were indeed forested to a much higher degree then they are today, though it is a sub-humid forest and significantly drier then the eastern rainforest zone. While there is no clear picture how much of the central highlands were forested, it is evident that human encroachment has severely altered the region (Burney 1997; Vågen 2006). The existing grasslands were

expanded, the original grasses replaced with 3-4 species of non-endemic grasses with the main tree now being eucalyptus. Forested areas in the highlands burned to create grazing lands. The highlands now consist of vast lands devoid of diversity.

While few biogeographical trends can accurately be described across the island, it is obvious that the diversity for this group is higher then expected. However, fragmented collecting and the lack of all life stages also inhibit conclusive statements regarding any trends regarding location, habitat type and elevation. Travel is difficult across the island, therefore, collection is constrained by the allotted time. Further investigation into the central highlands and the remaining fragments there as well as the northern forested regions that have not been collected in 60 years, and then only sparsely, is needed to truly have a clearer picture of the ecological trends that might be present within the group.

Conservation Issues

Located 400 km from the eastern coast of South Africa, Madagascar vibrantly illustrates the effects of geographic isolation. Separation from Africa 165 million years ago led to an explosion in biodiversity and to levels of endemism (Plants 89%; Mammals 93%; Birds 58%; Reptiles 96%; Amphibian 99%) virtually unmatched by any other biotic region (Goodman and Benstead, 2003). The Ramsar Convention declared Madagascar a biological hotspot for aquatic diversity in 2005. Aquatic arthropods appear to exhibit parallel trends in diversity and endemism, yet most groups remain poorly studied (Elouard and Gibon, 2003). Ignorance about aquatic insects, a group containing many sensitive bioindicators, is especially problematic at this time of acute concern about Madagascar's water quality. With

habitat destruction due to deforestation occurring at an alarming rate, many of Madagascar's species will become extinct in the next few decades.

As seen on many islands, the arrival of humans on Madagascar approximately 2000 years ago had devastating effects on the diversity of the island (Burney 1997). Within a short time the island's endemic megafauna became extinct, including the famous elephant bird, pygmy hippos and over a dozen species of giant lemurs (Burney 1997). One study done in the highlands showed that forest cover, which was at 8,060 ha in 1972, was down to 4,278 ha in 2001. While this does not reflect what has happened everywhere across the island, it does show the pressure put on the ecosystems due to the ever changing and intensifying needs of the population (Vagen 2006). Currently only 5% of the original landscape remains untouched across the island. As conservators race to protect the remaining forest, they are faced with the needs of the local people, who burn the forest to plant rice, their main source of nutrition and income, economic interests in the form of large, international corporations that raze the land for its valuable natural resources (trees and minerals), and a rapidly growing population within a turbulent government regime.

Stuckenberg (1958) expressed great concern at the probable loss of diversity due to deforestation and his concerns were justified. Several of Stuckenberg's collection sites from his 1956 – 1957 expedition have been cleared for agricultural use and are no longer suitable habitat for blephariceridsand other aquatic taxa. This is eloquently illustrated by Andringitra National Park. In correspondence to one of us (GWC), Stuckenberg spoke glowingly of the waterfall site at ANP, describing lush primary forest, lemurs calling in the mist, and a rich diversity of aquatic flies. Among the latter were several specimens of the only non-edwardsinine blephariceridknown from Madagascar. Additional material of this

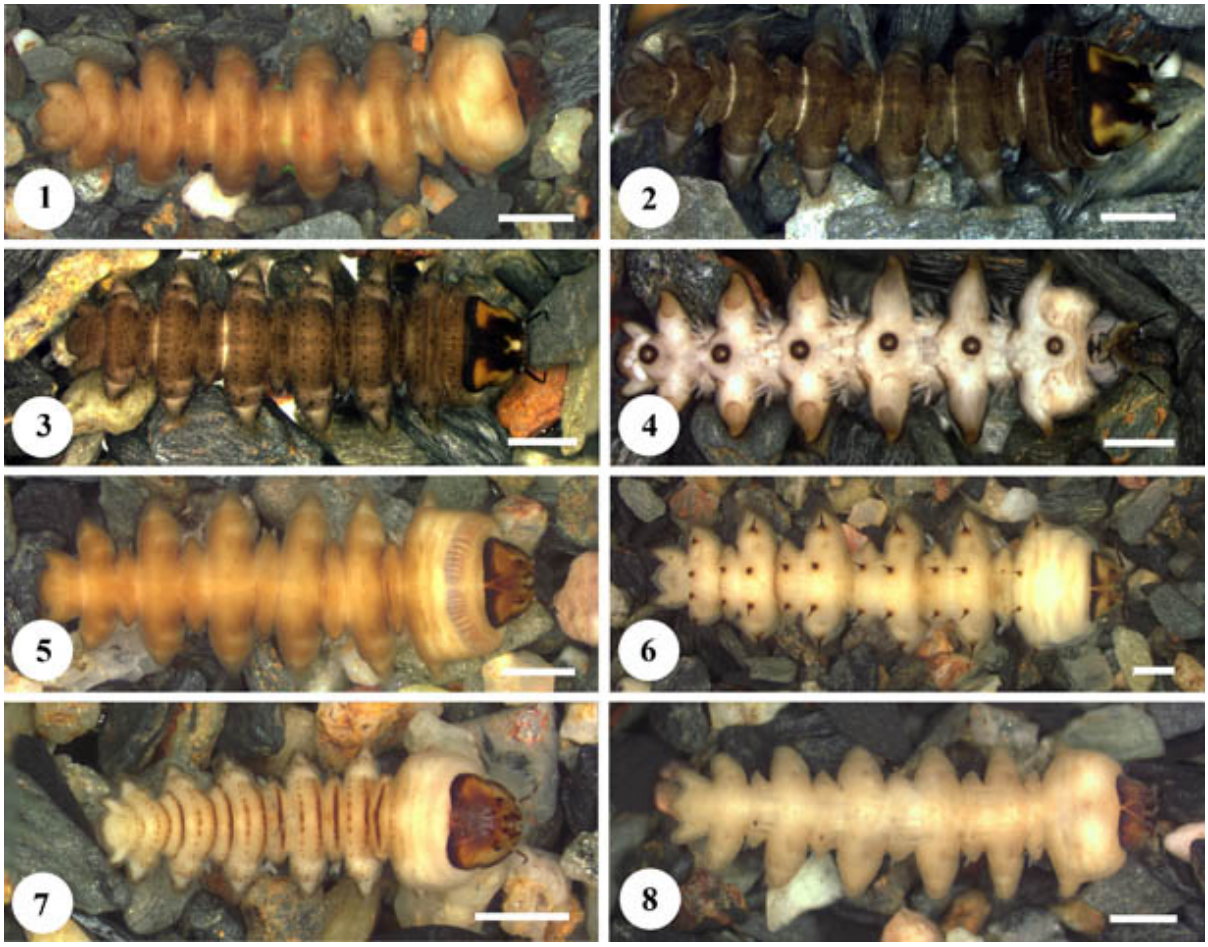
blephariceridid, which remains undescribed and unnamed, was a primary objective of a 2007 visit to ANP. Unfortunately, upon arrival in 2007, we neither observed nor heard lemurs, nearly all of the forest had been cleared, and grazing was apparent in the remaining secondary forest. Furthermore, the overall aquatic diversity was much lower than expected, and no specimens of the non-edwardsinine blephariceridid were found. Despite the destruction at this particular site, we are hopeful that streams and waterfalls farther into the park remain relatively pristine and represent promising potential habitats for net-winged midges. We are also hopeful that these areas will be surveyed for net-winged midges as soon as possible, partly to determine if the non-edwardsinine species still exists or has been extirpated by deforestation of the lower reaches of ANP's rivers.

Andringitra is not the only park that has suffered significantly due to habitat destruction. For example, in the time between collection of new material in 2006 and this publication at least one site was destroyed at VSR to clear a 100m wide tract of forest for a mineral pipeline to the coast. That tract contained one new species, also collected at a nearby location in 1958 by Stuckenberg. Now, due to habitat destruction, this new species may be extinct.

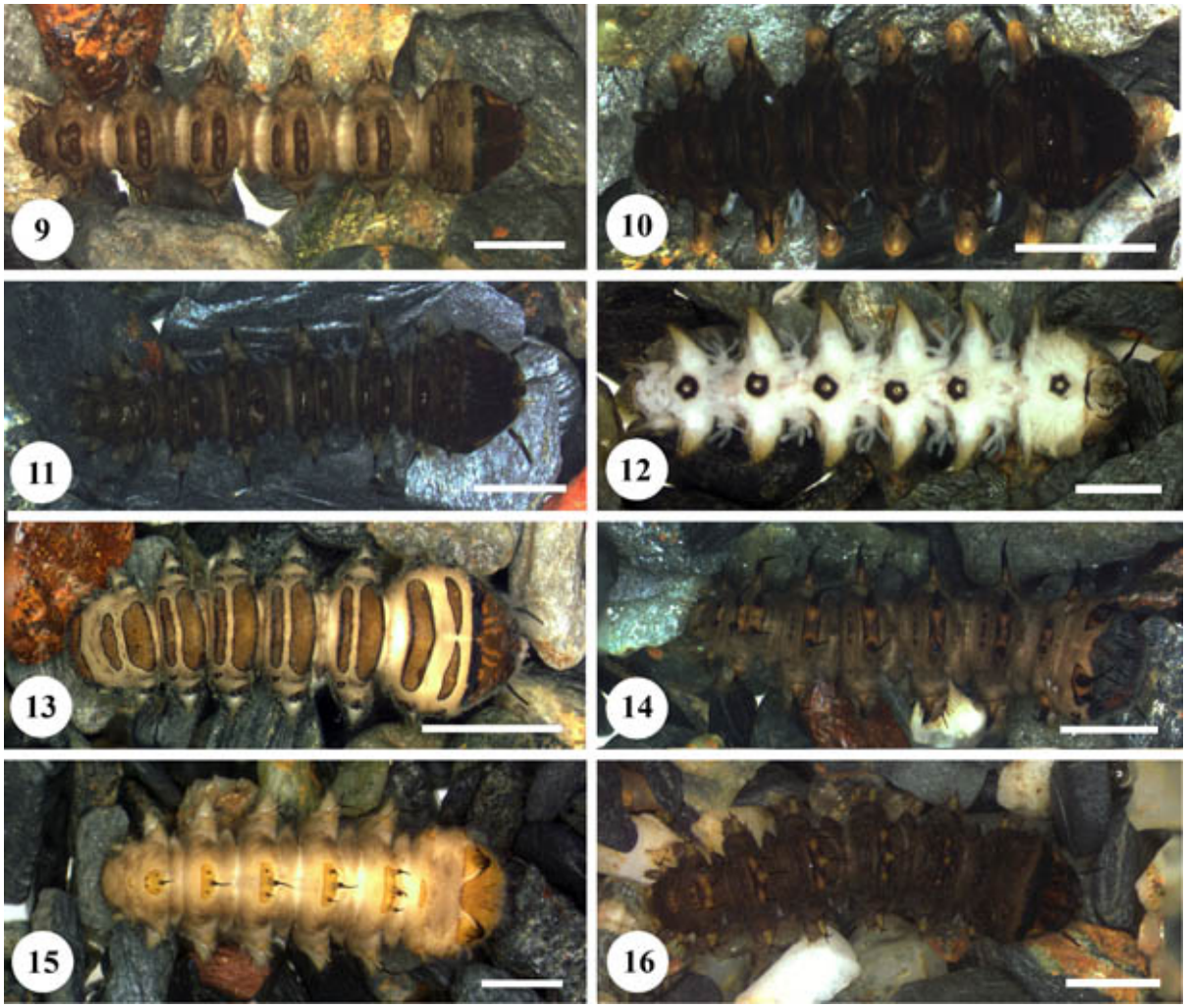
Of special note is the presence of blephariceridids at Ambohitantely Special Reserve (ASR). The reserve is a small forest fragment in the central highlands, approximately 1,500 meters elevation and surrounded by kilometers of low-diversity grasslands. Because all known blephariceridids were from Madagascar's eastern forests, we did not expect to find these flies at ASR. However, six species were collected during several expeditions from 2004-2007. Streams immediately outside the reserve yielded no specimens. This site, more than any other, indicates that the family was once more widespread than originally thought,

but that deforestation over the last several centuries has decreased their habitat to small fragments.

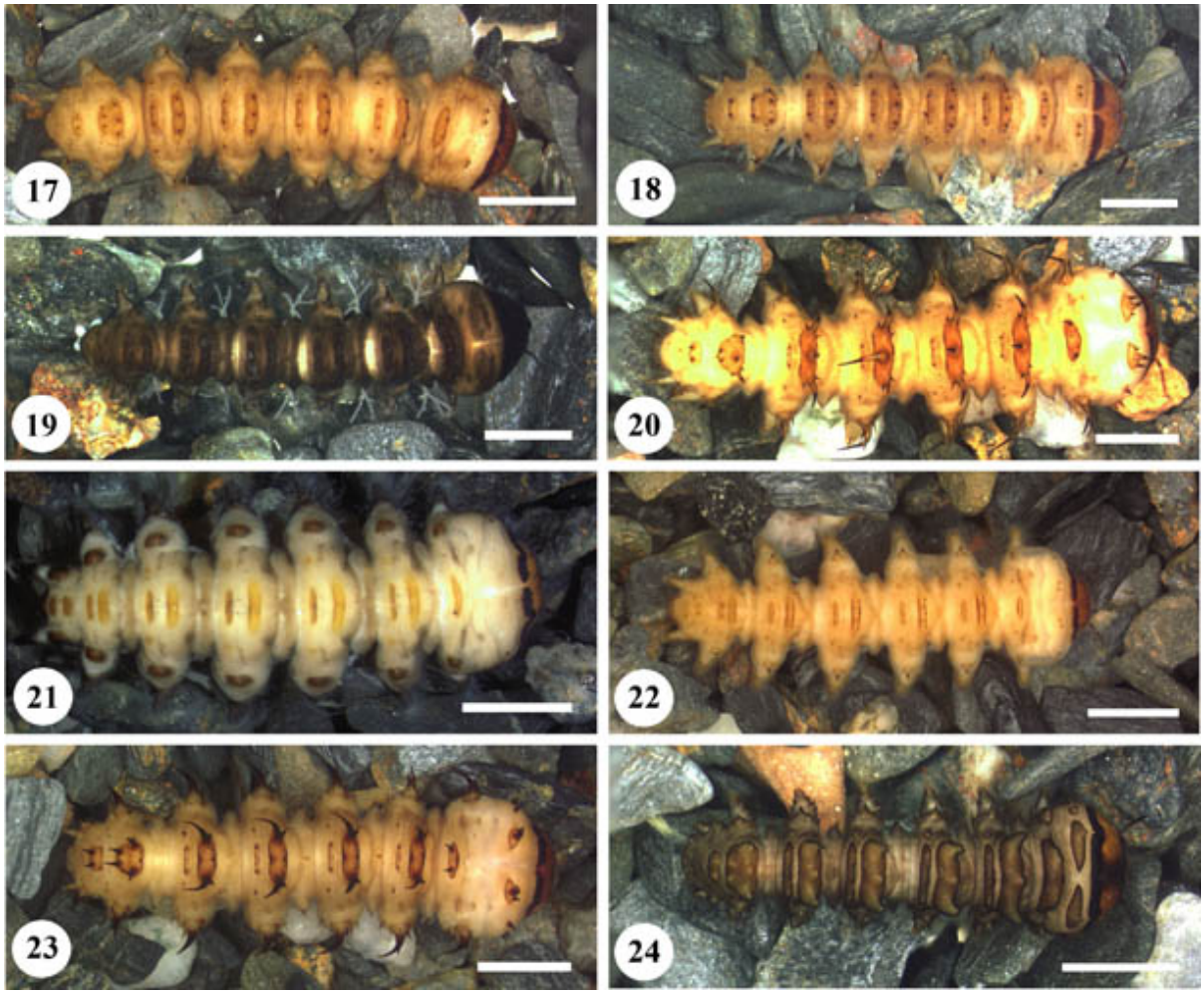
This ongoing, rapid destruction of habitat across the country requires the aggressive cataloguing of existing biodiversity in Madagascar's remaining forests. It also provides a sound basis for providing names to all new species, within the boundaries of species delimitation, regardless of life stage. Many local NGOs and international groups have worked tirelessly to preserve the remaining beauty of the island. Taxonomists are desperately needed to assist this effort, by continuously updating and illustrating the astounding diversity that remains on this uniquely magnificent island.



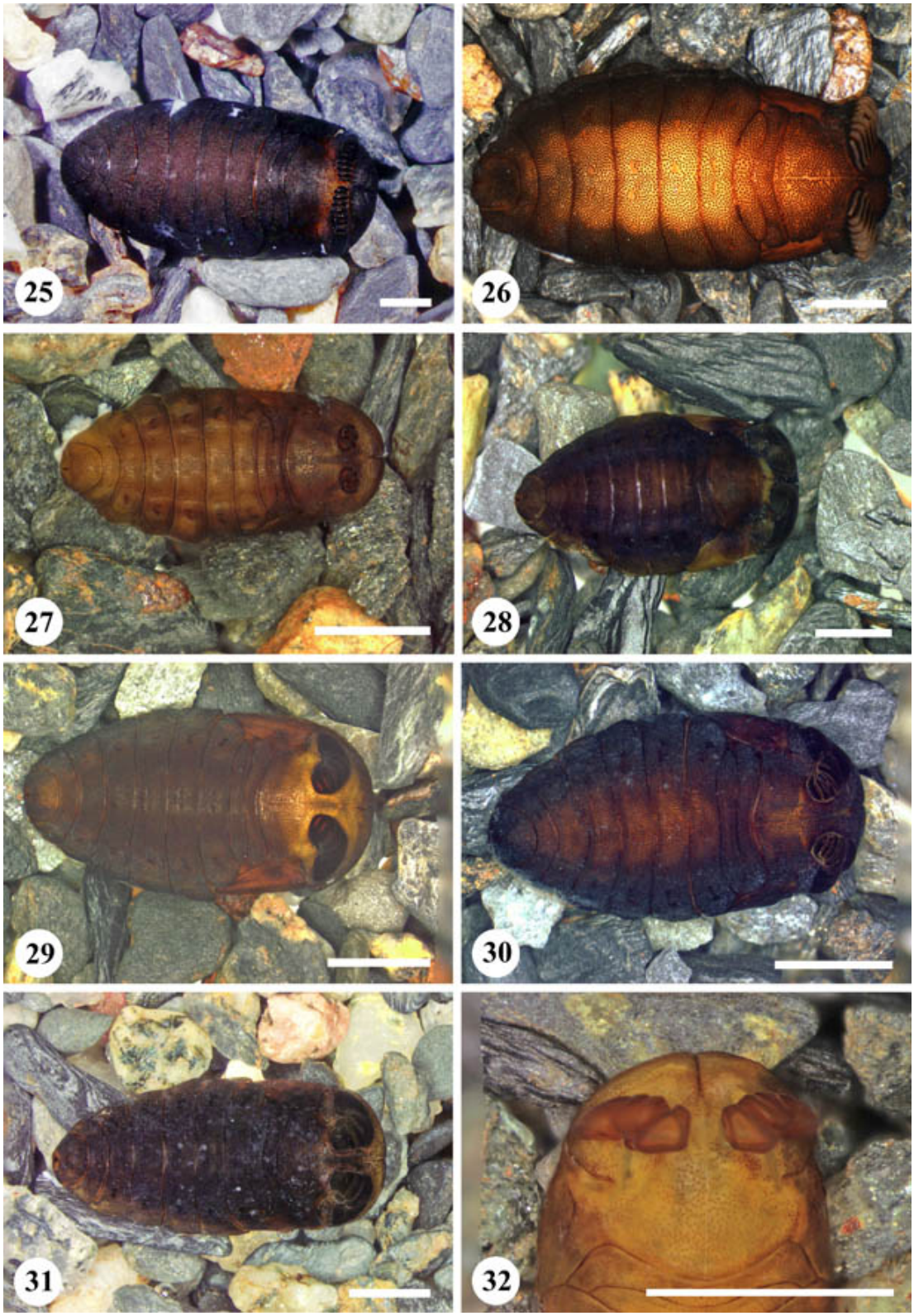
Figs 1-8. Light micrographs of larval *Paulianina*, dorsal and ventral views. 1, *P. ingens*, dorsal view. 2, *P. umbra*, dorsal view. 3, *P. umbra*, dorsal view. 4, *P. umbra*, ventral view. 5, *P. umbra*, dorsal view. 6, *P. lehibe* dorsal view. 7, *P. ratsipika*, dorsal view. 8, Species J, dorsal view. Scale bars = 1mm.



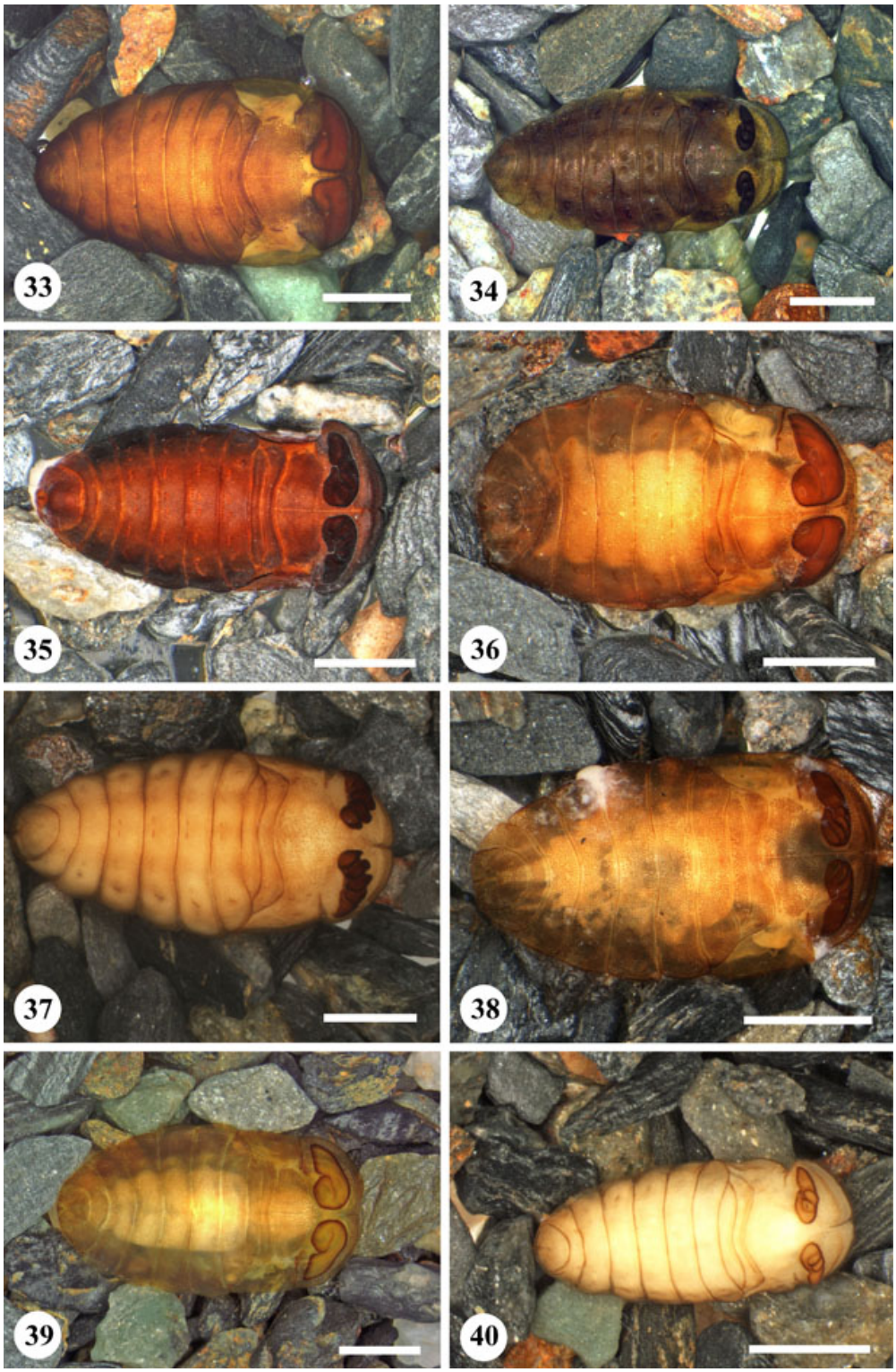
Figs 9-16. Light micrographs of larval *Eupaulianina*, dorsal and ventral views. 9, *E. pamela*, morpho-type one, dorsal view. 10, *E. pamela*, morpho-type two, dorsal view. 11, *E. alexanderi*, dorsal view. 12, *E. alexanderi*, ventral view. 13, *E. rivalis*, dorsal view 14, *E. silva*, dorsal view. 15, *E. tsilobe*, dorsal view. 16, *E. telofantsy*, dorsal view. Scale bars = 1mm.



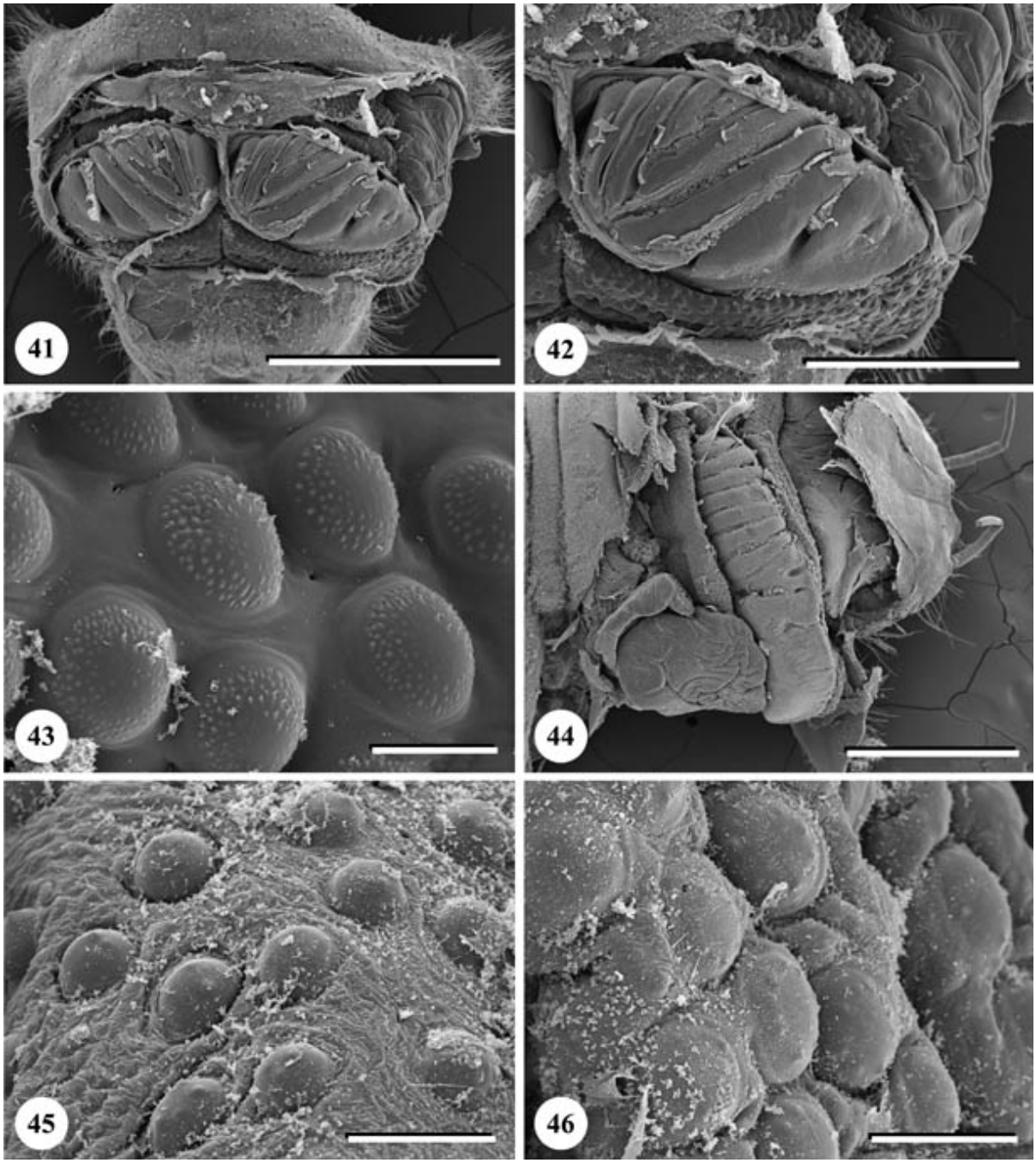
Figs. 17-24. Light micrographs of larval *Eupaulianina*. 17, *E. borivody*. 18, *E. korontantsilo*. 19, *E. marobotsin*. 20, *E. marangitsilo*. 21, *E. vohimalama*. 22, *E. botsimpatsy*. 23, *E. ratsilo*. 24, *E. tandroka*. Scale bars = 1mm.



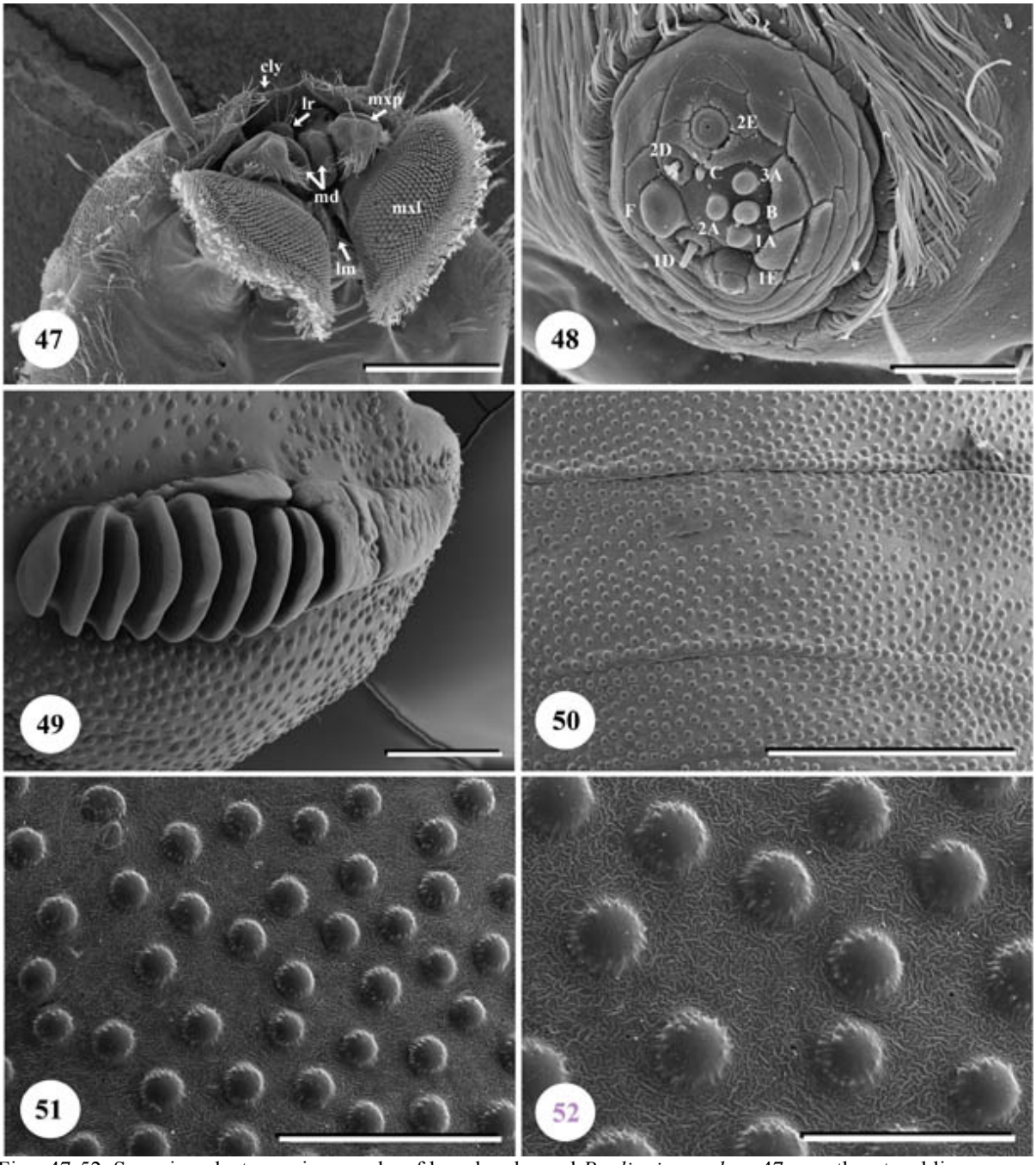
Light micrographs of pupal *Paulianina* (Figs. 25-26) and *Eupaulianina* Figs. 27-32), dorsal view. 25, *P. umbra*. 26, *P. lehibe*. 27, *E. pamela*. 28, *E. alexanderi*. 29, *E. silva*. 30, *E. tsilobe*. 31, *E. telofantsy*. 32, *E. borivody*. Scale bars = 1mm.



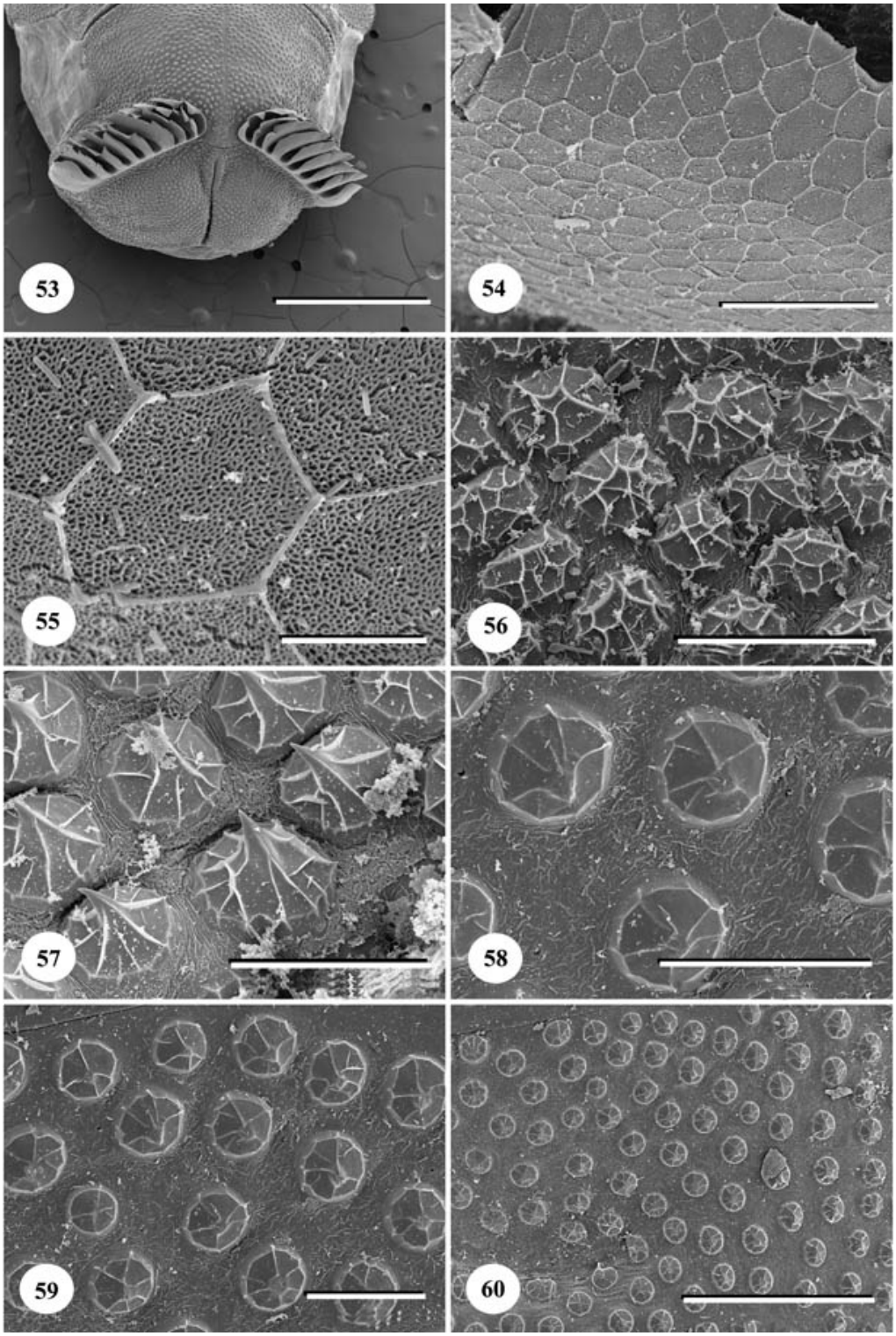
Light micrographs of pupal *Eupaulianina*, dorsal view. 33, *E. korontantsilo*. 34, *E. marobotsin*. 35, E. species K. 36, E. species L. 37, E. species M. 38, E. species N. 39, E. species O, 40. E. species P. Scale bars = 1mm.



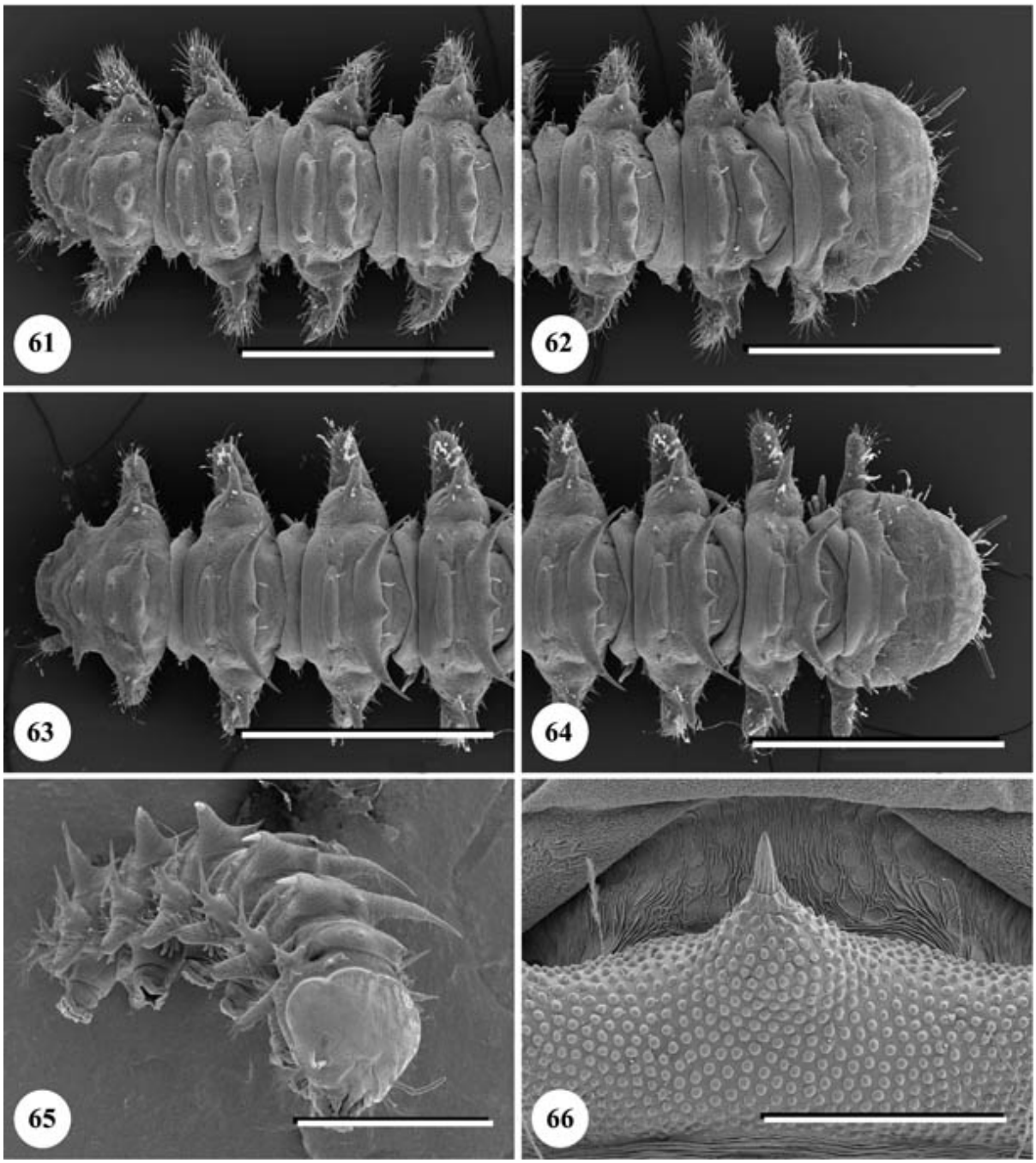
Figs. 41-46. Scanning electron micrographs of *Paulianina* pupae (dissected from mature larvae). 41-43, *P. ingens*. 44-46, *P. umbra*. 41, respiratory organ . 42, left respiratory organ. 43, microsculpture on 3rd abdominal segment. 44, respiratory organ. 45, microsculpture on 3rd abdominal segment. 46, microsculpture on 3rd abdominal segment. Scale bars = 20µm (Figs. 43, 45-46), 500µm (Fig. 44), 600µm (Fig. 42), 1000µm (Fig. 41).



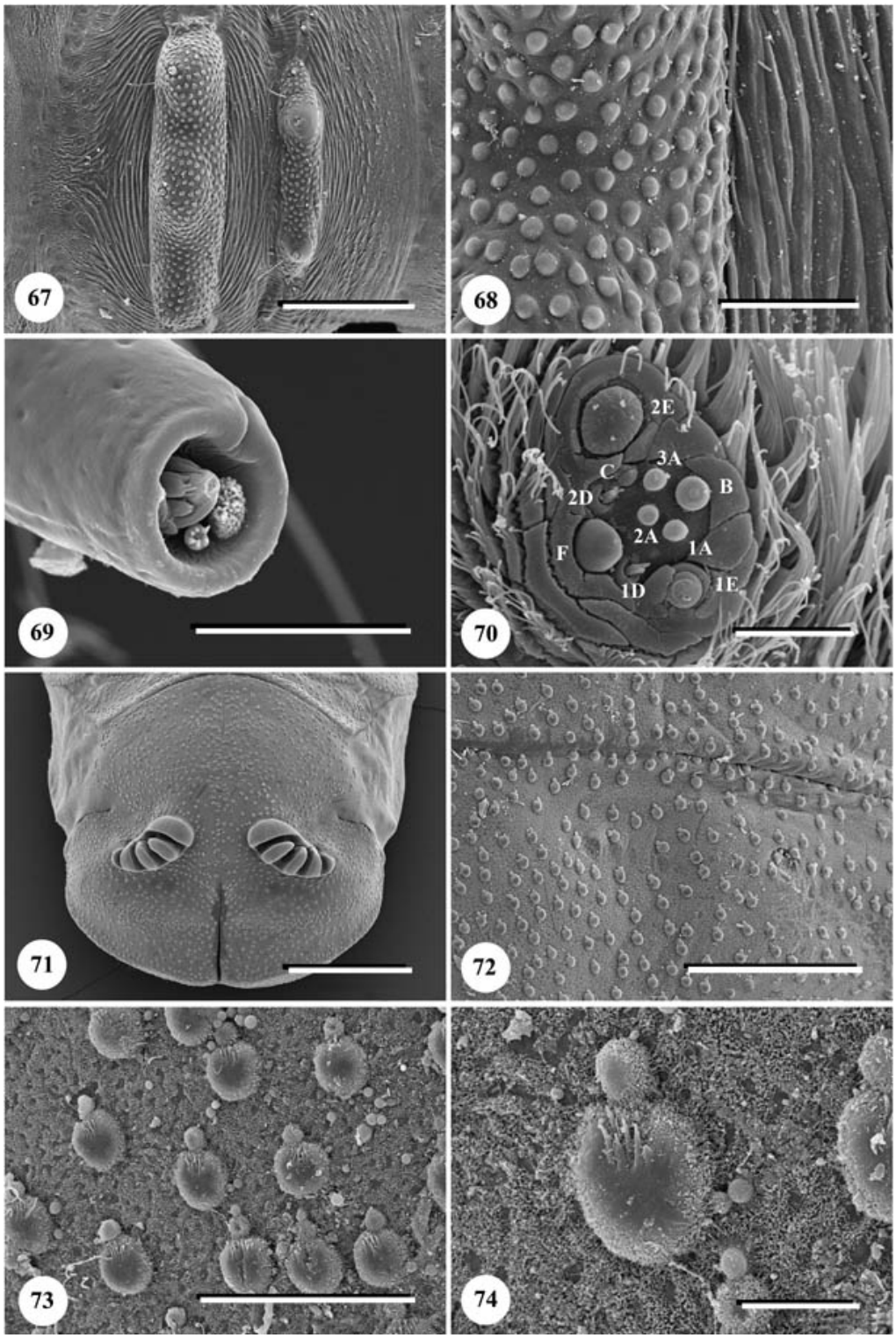
Figs. 47-52. Scanning electron micrographs of larval and pupal *Paulianina umbra*. 47, mouthparts, oblique ventral view. 48, larval maxillary palpus. 49, pupal respiratory organ. 50-52, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Fig. 48), 50µm (Fig. 52), 100µm (Fig. 51), 200µm (Figs. 47, 49), 500µm (Fig. 50). (abbreviations: lm = labium; lr = labrum; md = mandible; mxl = maxilla; mxp = maxillary palpus; 1A, 2A, 3A, B, C, 1D, 2D, 1E, 2E, F = maxillary palpus sensilla).



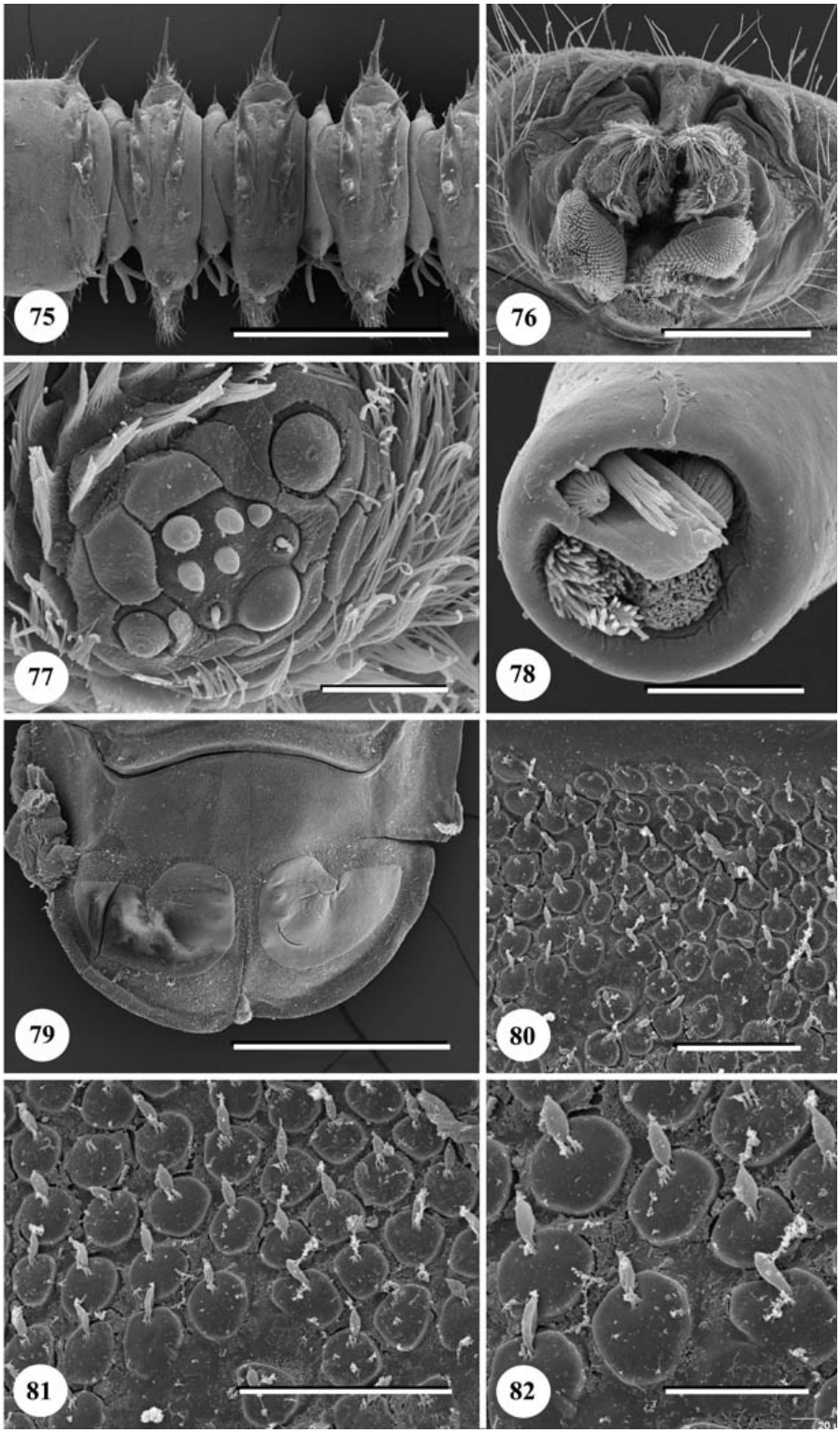
Figs. 53-60. Scanning electron micrographs of larval and pupal *Paulianina lehibe*. 53, anterior of pupa, showing respiratory organs, dorsal view. 54, microstructure of pupal respiratory organ. 55, microstructure of pupal respiratory organ structure. 56, pupal abdominal microsculpture near respiratory organ. 57-59, pupal abdominal microsculpture, tergit III. 60, pupal microsculpture (dissected from mature larva) tergite III. Scale bars = 10µm (Fig. 55), 50µm (Figs. 54, 57-60), 100µm (Fig. 56), 200µm, 1000µm (Fig. 53).



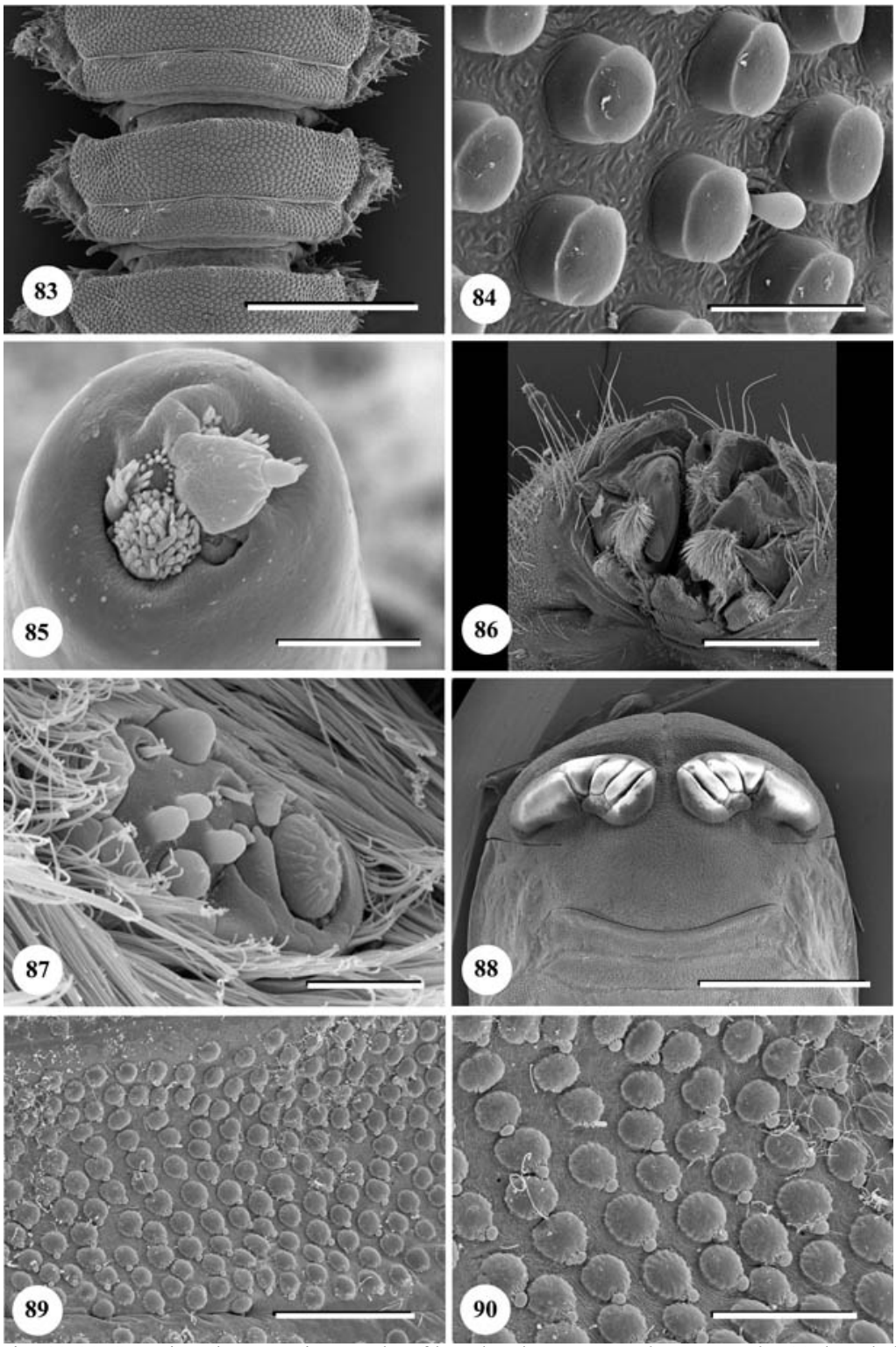
Figs. 61-66. Scanning electron micrographs of larval and pupal *Eupaulianina pamela.* 61, first morpho-type of larva, posterior, dorsal. 62, first morpho-type of larva, anterior, dorsal. 63, second morpho-type of larva, posterior, dorsal. 64, second morpho-type of larva, anterior, dorsal. 65, anterodorsal view of second larval morpho-type. 66, microsculpture of second larval morpho-type. Scale bars = 200µm (Fig. 66), 1000m (Figs. 61-64), 1mm (Fig. 65).



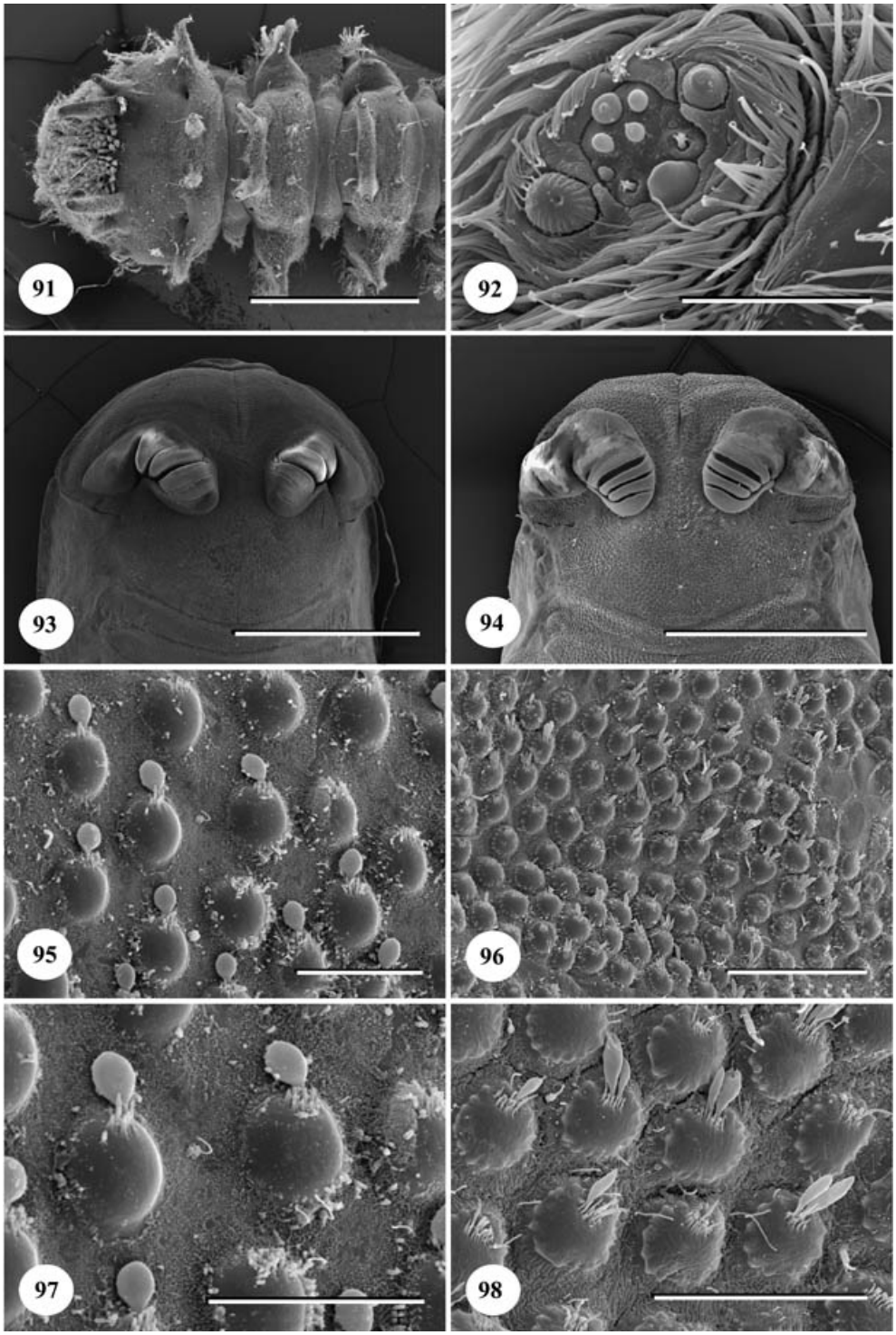
Figs. 67-74. Scanning electron micrographs of larval and pupal *Eupaulianina. E pamela*. 67, microsculpture of first larval morpho-type. 68, microsculpture of first larval morpho-type. 69, larval antennae. 70, larval maxillary palpus. 71, anterior view of pupa, including respiratory organs. 72--74, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Figs. 70, 74), 20µm (Fig. 69), 50µm (Figs. 68, 73), 200µm (Figs. 67, 72), 500µm (Fig. 71). (abbr: 1A, 2A, 3A, B, C, 1D, 2D, 1E, 2E, F = mxp sensilla)



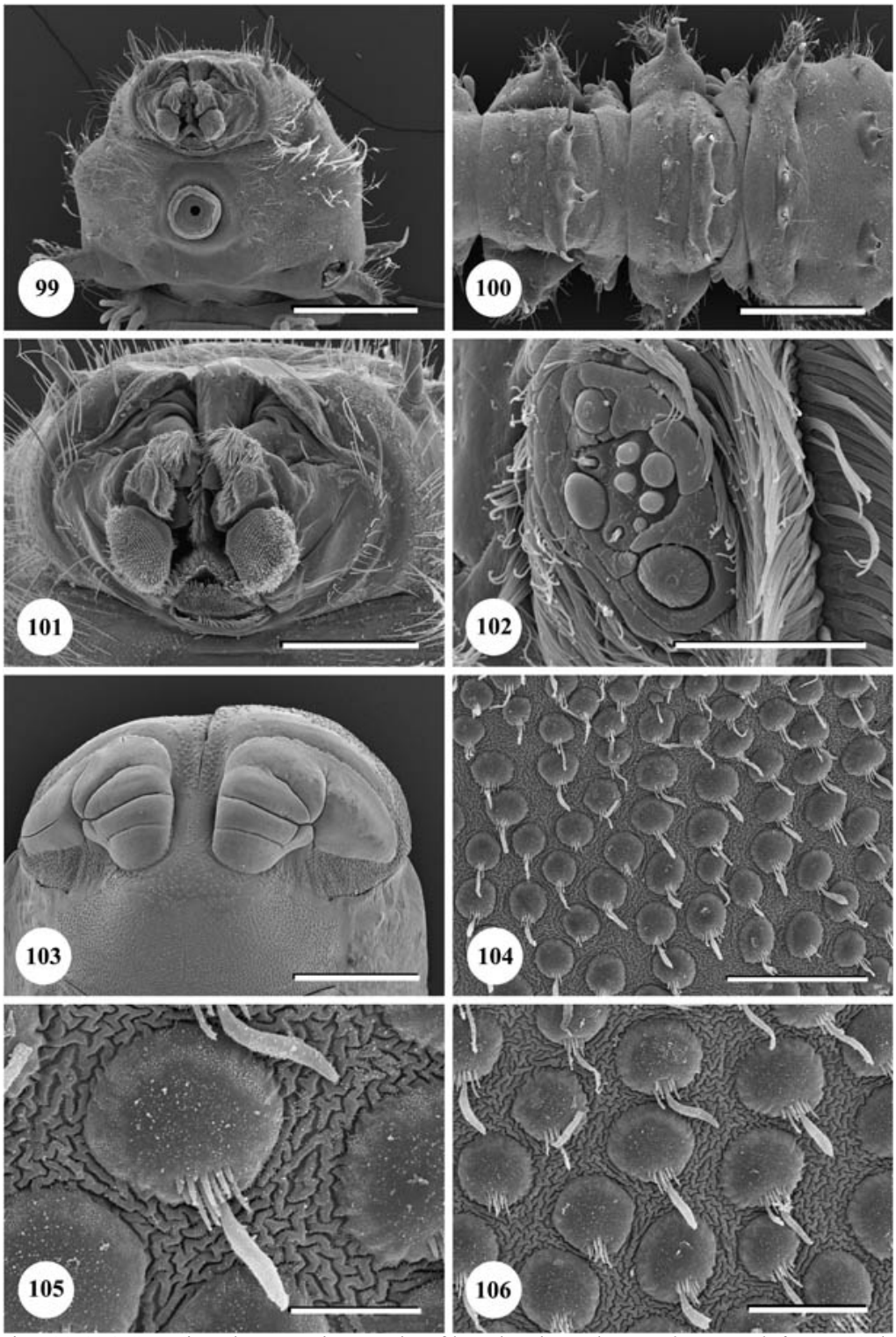
Figs. 75-82. Scanning electron micrographs of larval and pupal *Eupaulianina alexanderi*. 75, dorsal view larva. 76, larval mouthparts. 77, larval maxillary palpus. 78, larval antennae. 79, pupal respiratory organ. 80-82, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Figs. 77, 78), 20µm (Fig. 82), 50µm (Figs. 80, 81), 200µm (Fig. 76), 1000m (Figs. 75, 79).



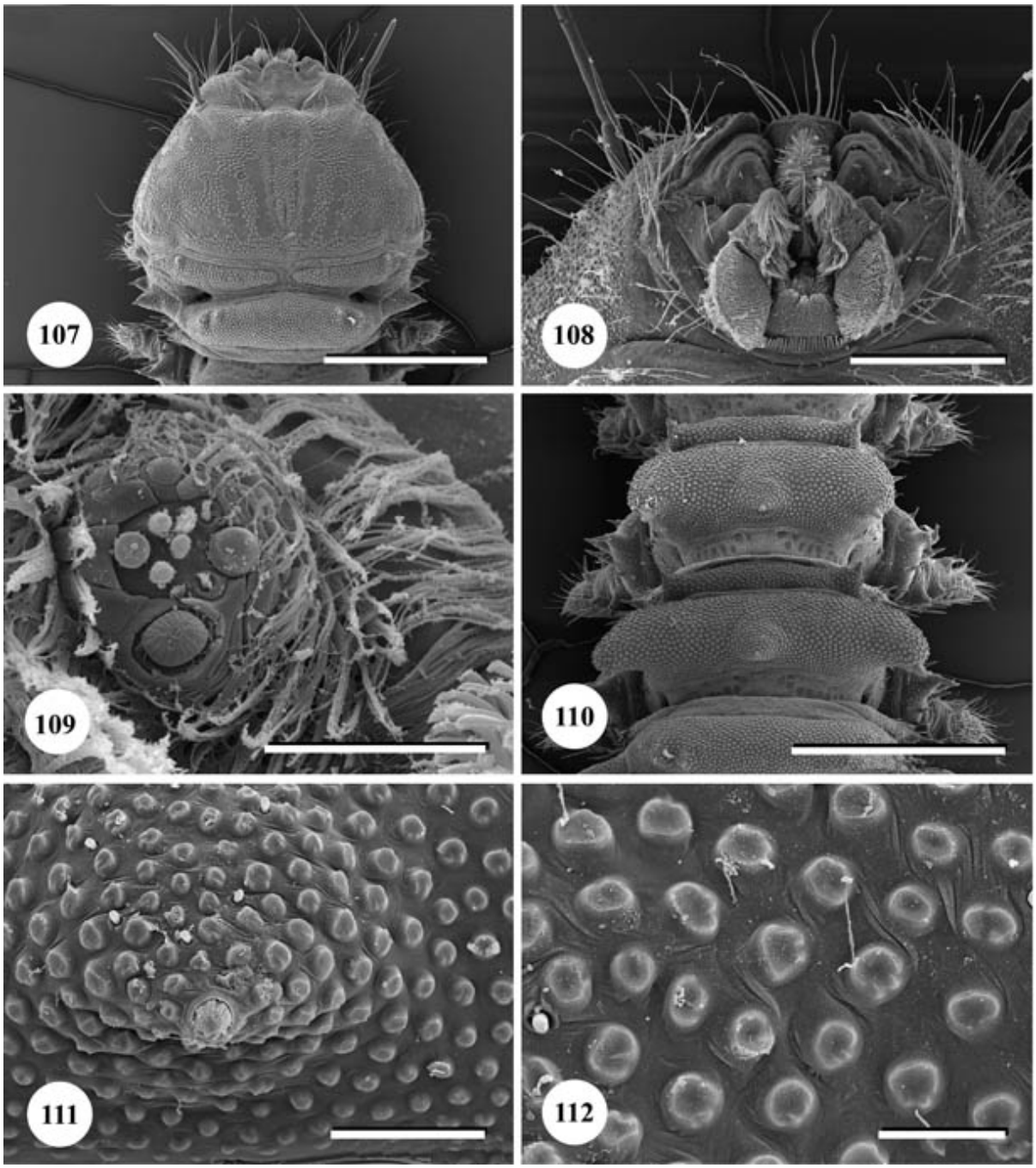
Figs 83-90. Scanning electron micrographs of larval and pupa *Eupaulianina rivalis*. 83, larval abdominal segments 3-5, dorsal view. 84, larval microsculpture, segment 3, dorsal view. 85, larval antennae, apical sensilla. 86, larval mouthparts, oblique frontal view. 87, larval maxillary palpus. 88, anterior of pupa showing respiratory organs, dorsal view. 89-90, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Figs. 85, 87), 20µm (Fig. 84), 60 µm (Fig. 90), 200 µm (Fig. 86), 500 µm (Fig. 83), 1000µm (Fig. 88).



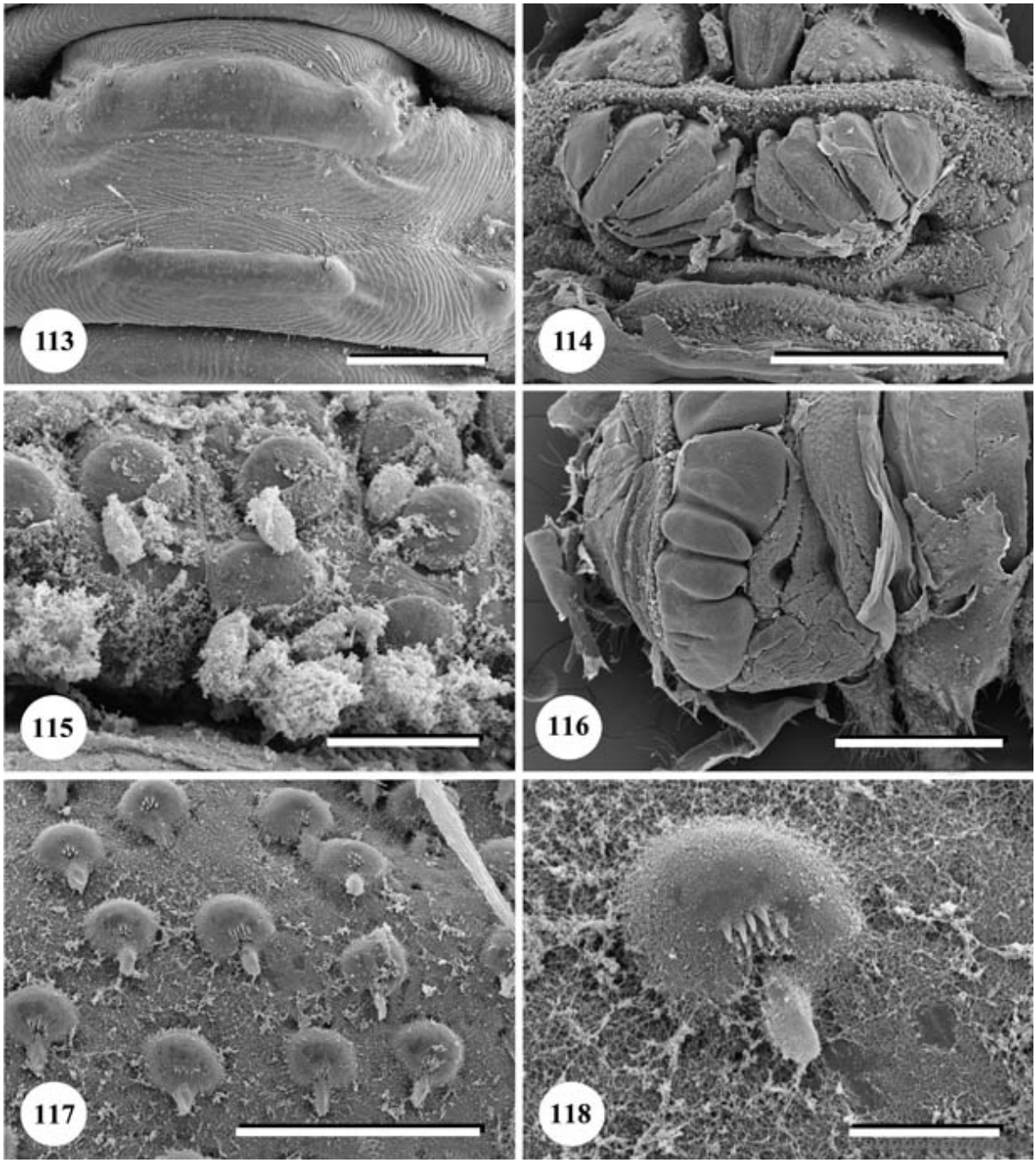
Figs. 91-98. Scanning electron micrographs of larval and pupal *Eupaulianina silva* (Figs. 91, 93, 95, 97), *Eupaulianina tsilobe* (Figs. 92, 94, 96, 98). 91, larval cephalothorax and abdominal segments 2-3, dorsal view. 92, larval maxillary palpus. 93, anterior of pupa showing respiratory organs, dorsal view. 94, anterior of pupa showing respiratory organs, dorsal view. 95-98, pupal abdominal microsculpture, tergite III. Scale bars = 20µm (Figs. 92, 95, 97), 50µm (Fig. 98), 100 µm (Figs. 96), 1000µm (Figs. 91, 93, 94).



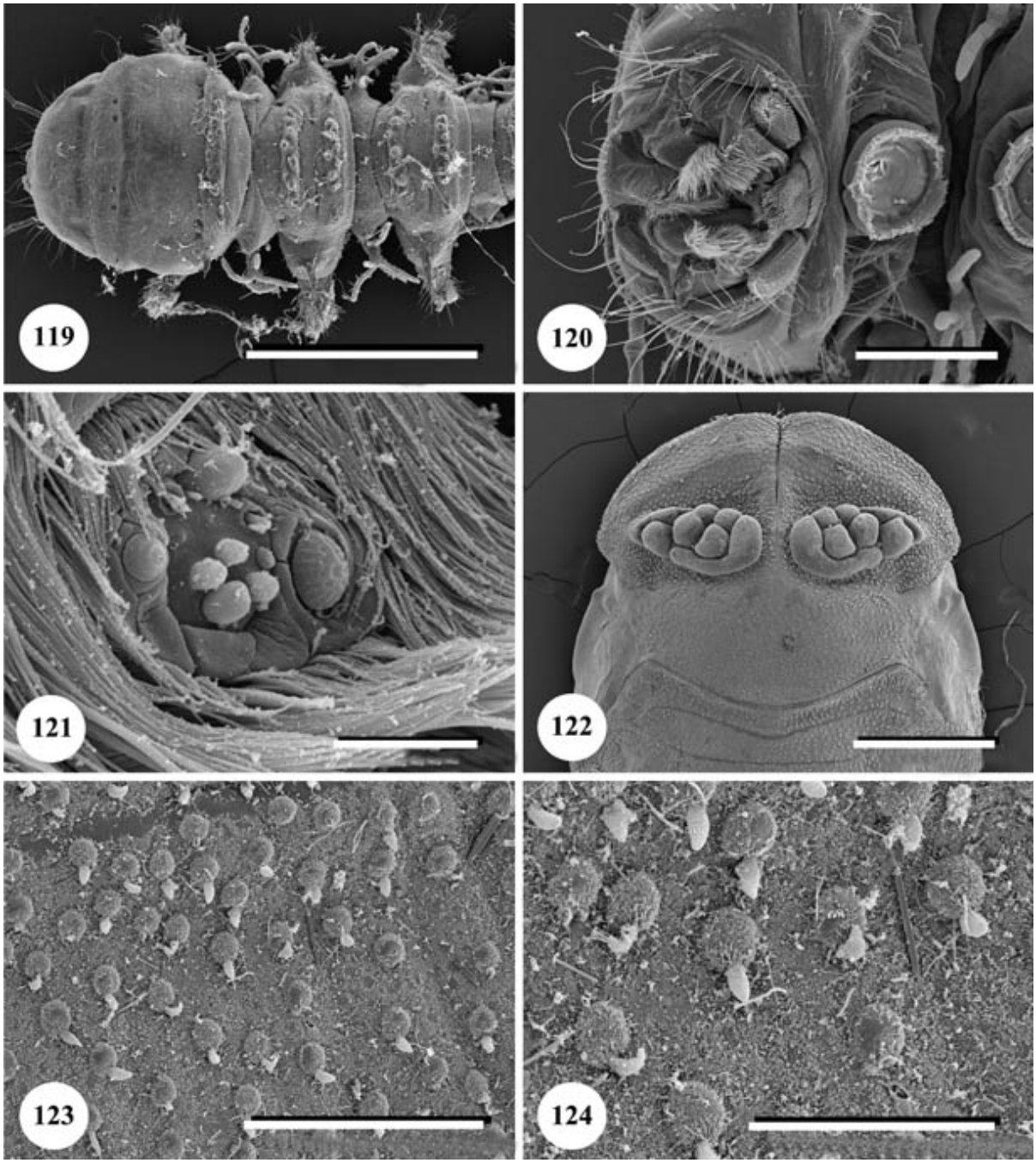
Figs. 99-106. Scanning electron micrographs of larval and pupal *Eupaulianina telofantsy*. 99, larval cephalothorax, ventral view. 100, larval abdominal segments II-III, dorsal view. 101, larval mouthparts, ventral view. 102, larval maxillary palpus. 103, anterior of pupa showing respiratory organs, dorsal view. 104-106, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Fig. 105), 20µm (Figs. 102, 106), 50µm (Fig. 104), 200 µm (Fig. 101), 500 µm (Fig. 99, 100, 103).



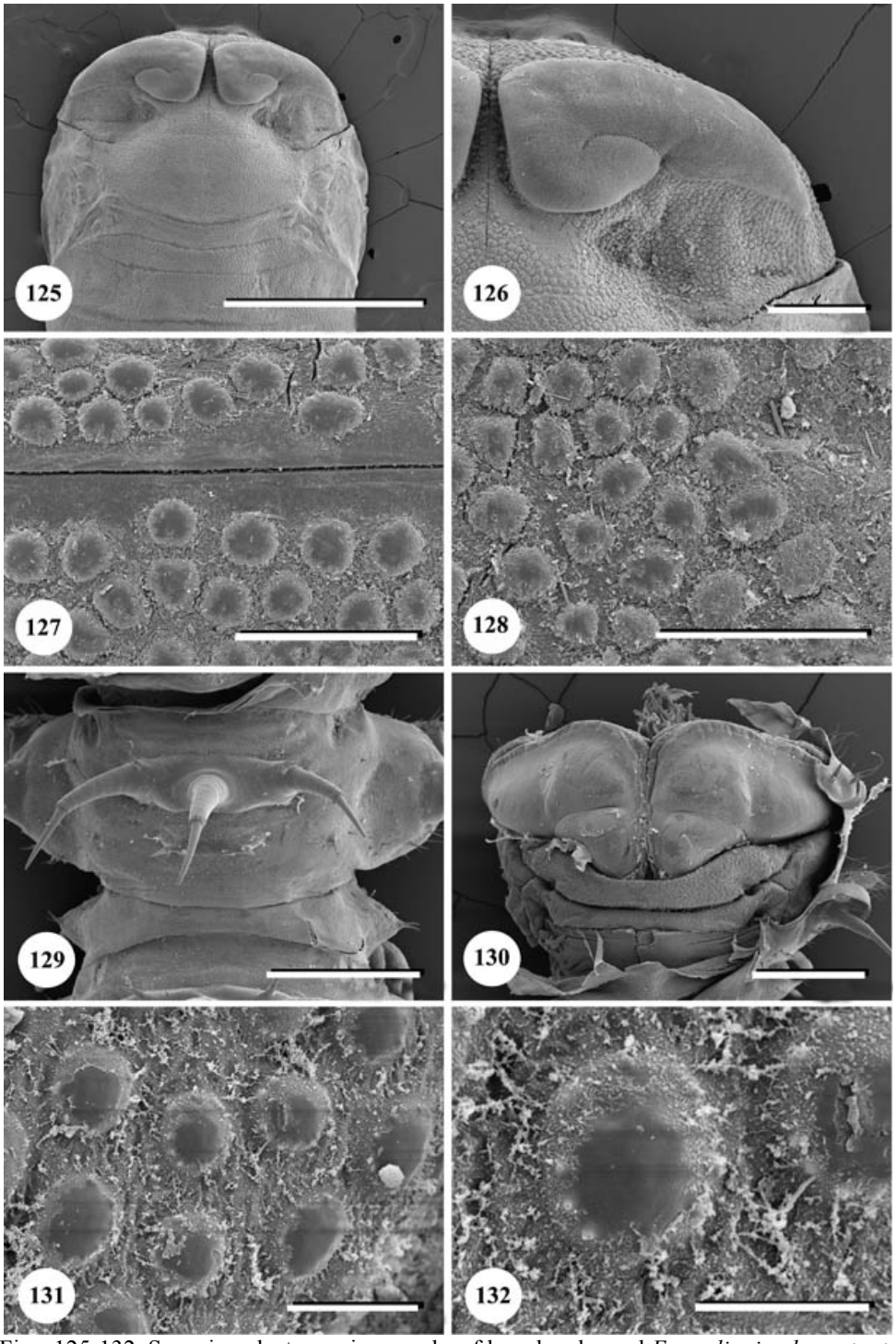
Figs. 107-112. Scanning electron micrographs of larval and pupal *Eupaulianina tandroka.* 107, larval cephalothorax, dorsal view. 108, larval mouthparts. 109, larval maxillary palpus. 110, larval abdomen, dorsal view (anterior = bottom of image). 111, larval microsculpture, dorsum of segment II. 112, larval microsculpture, dorsum of segment II. Scale bars = 20µm (Figs. 109, 112), 50µm (Figs. 111). 200µm (Figs. 108), 500µm (Figs. 107, 110).



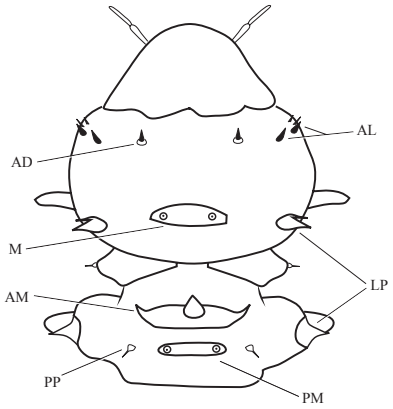
Figs. 113-118. Scanning electron micrographs of larval and pupa *Eupaulianina vohimalama* (Figs. 113-115) and *Eupaulianina borivody* (Figs. 116-118). 113, larval abdominal segment III, dorsal view. 114, pupal respiratory organs dissected from mature larva. 115, pupal microsculpture tergite I, dissected from mature larva. 116, pupal respiratory organs dissected from mature larva. 117-118, pupal microsculpture tergite 1, dissected from mature larva. Scale bars = 10µm (Fig. 118), 20µm (Fig. 115), 50µm (Fig. 117). 200µm (Fig. 113), 500µm (Figs. 114, 116).

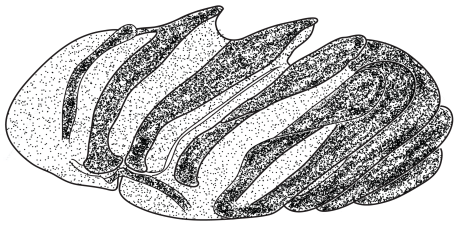


Figs. 119-124. Scanning electron micrographs of larval and pupal *Eupaulianina marobotsin.* 119, larval cephalothorax and abdominal segments II-III, dorsal view. 120, larval mouthparts. 121, larval maxillary palpus. 122, anterior of pupa showing respiratory organs. 123-124, pupal abdominal microsculpture, tergite III. Scale bars = 10µm (Fig. 121), 50µm (Fig. 124), 100µm (Fig. 123), 200µm (Fig. 120), 500µm (Fig. 122), 1000µm (Fig. 119).

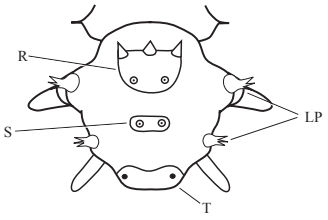


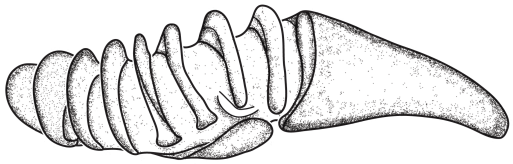
Figs. 125-132. Scanning electron micrographs of larval and pupal *Eupaulianina korontantsilo* (Figs. 125-128) and *Eupaulianina ratsilo* (Figs. 129-132). 125, anterior of pupa showing respiratory organs. 126, right pupal respiratory organ. 127-128, pupal abdominal microsculpture, tergite III 129, larval abdominal segment II. 130, pupal respiratory organs dissected from mature larva. 131-132, pupal microsculpture tergite I, dissected from mature larva. Scale bars = 10µm (Fig. 132), 20µm (Fig. 131), 50µm (Figs. 127, 128), 200µm (Fig. 126), 500µm (Figs. 129, 130), 1000µm (Fig. 125)





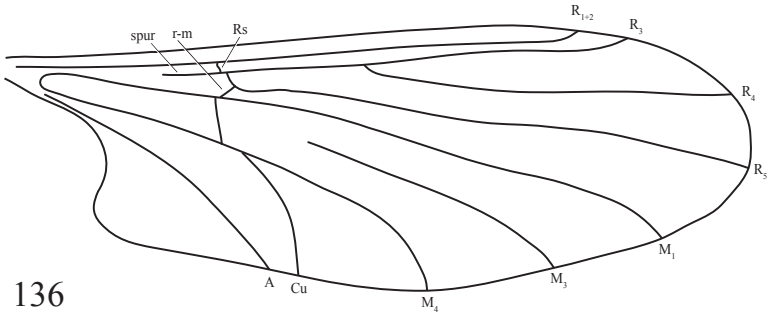
134



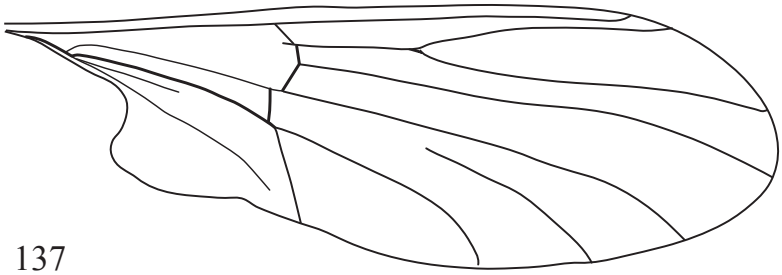


135

133

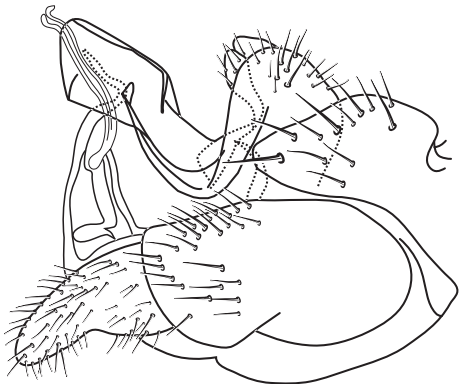


136

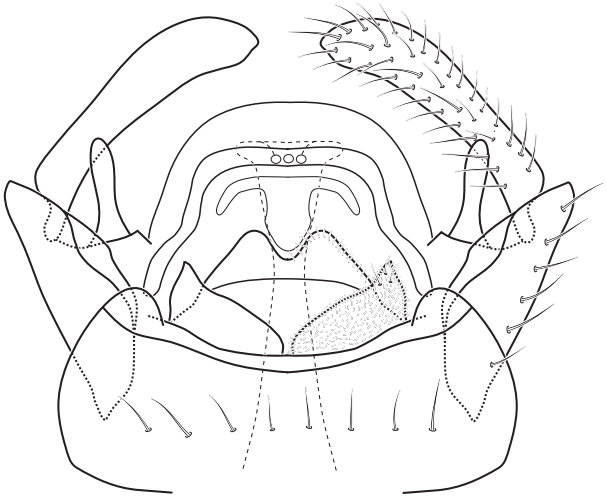


137

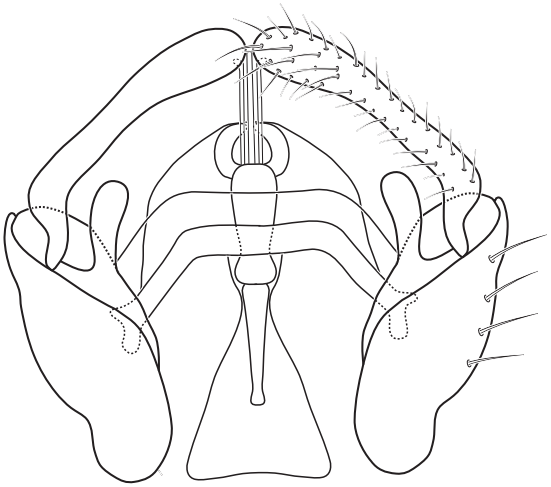
Figures 133-137. 133, Generalized chaetotaxy of *Eupaulianina* larvae. 134, *P. ingens* pupal respiratory organ. 135, *P. umbra* pupal respiratory organ. 136, *P. umbra,* wing venation . 137, *E. alexanderi* wing venation.



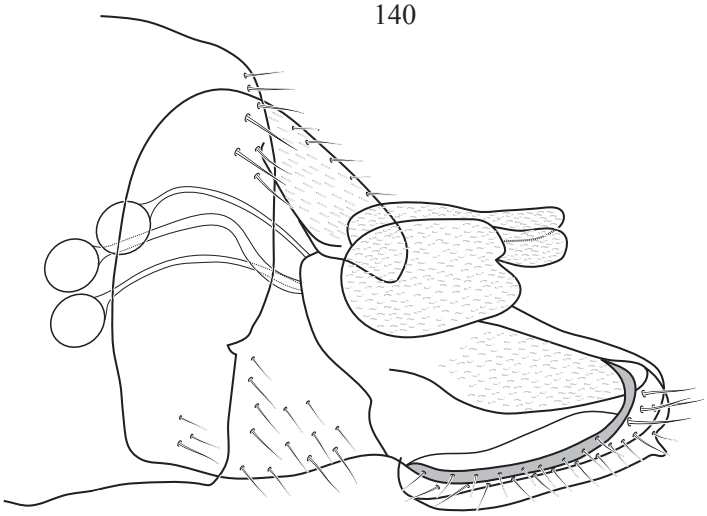
138



139

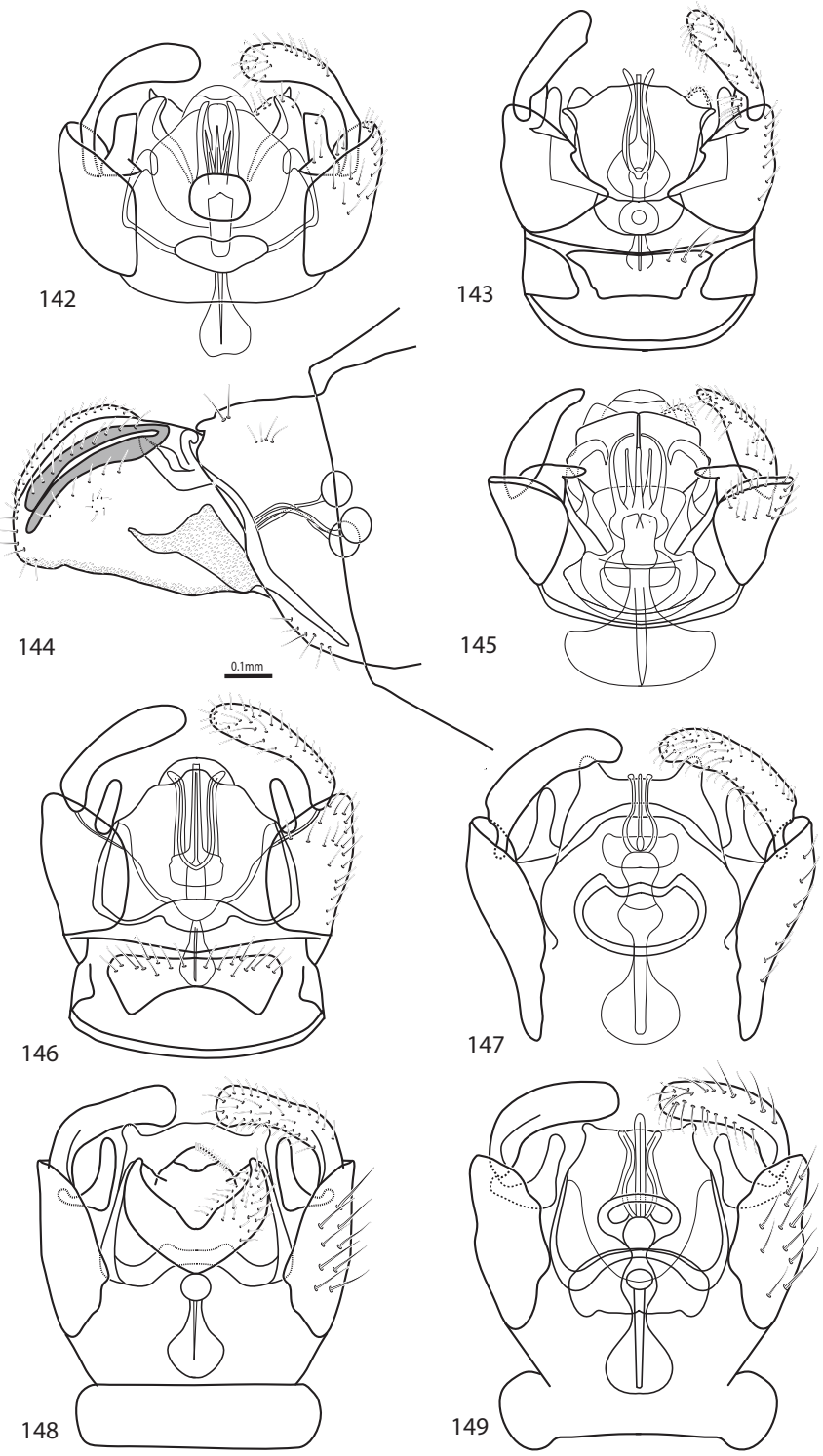


140



141

Figs. 138-141. 138, *P. ingens* male genitalia, lateral view. 139, *P. umbra* male genitalia, dorsal view. 140, *P. umbra* male genitalia, ventral view. 141, *P. umbra* female genitalia, lateral view.



Figures 142-149. 142, *E. pamela* male genitalia, ventral view. 143, *E.alexenaderi* male genitalia, ventral view. 144, *E. alexanderi* female genitalia, lateral view. 145, *E. rivalis* male genitalia, ventral view. 146, *E. silva* male genitalia, ventral view. 147, *E. tsilobe*, male genitalia, ventral view. 148, *E. fantsona*. male genitalia, dorsal view. 149, *E. fantsona*., male genitalia, ventral view. (Figs. 143, 145, 146 adapted from Stuckenberg 1958)

Figure 150. Bootstrap tree based on morphological characters of all life stages. Numbers above each node denote bootstrap support. (CI = 0.54; RI = 0.63)

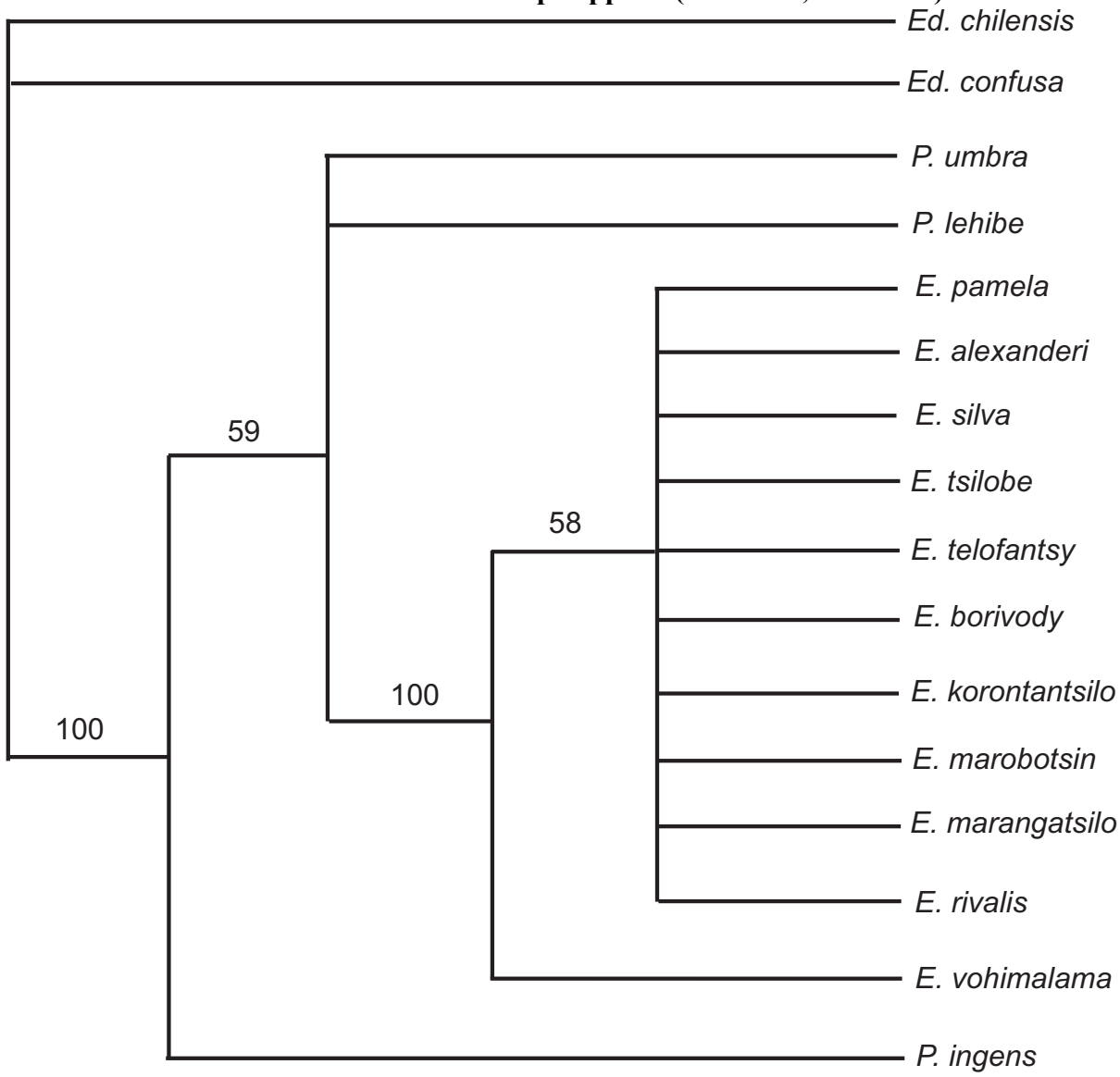


Figure 151. Bootstrap tree based on morphological characters of larvae and pupae. Numbers above each node denote bootstrap support. (CI = 0.65; RI = 0.78)

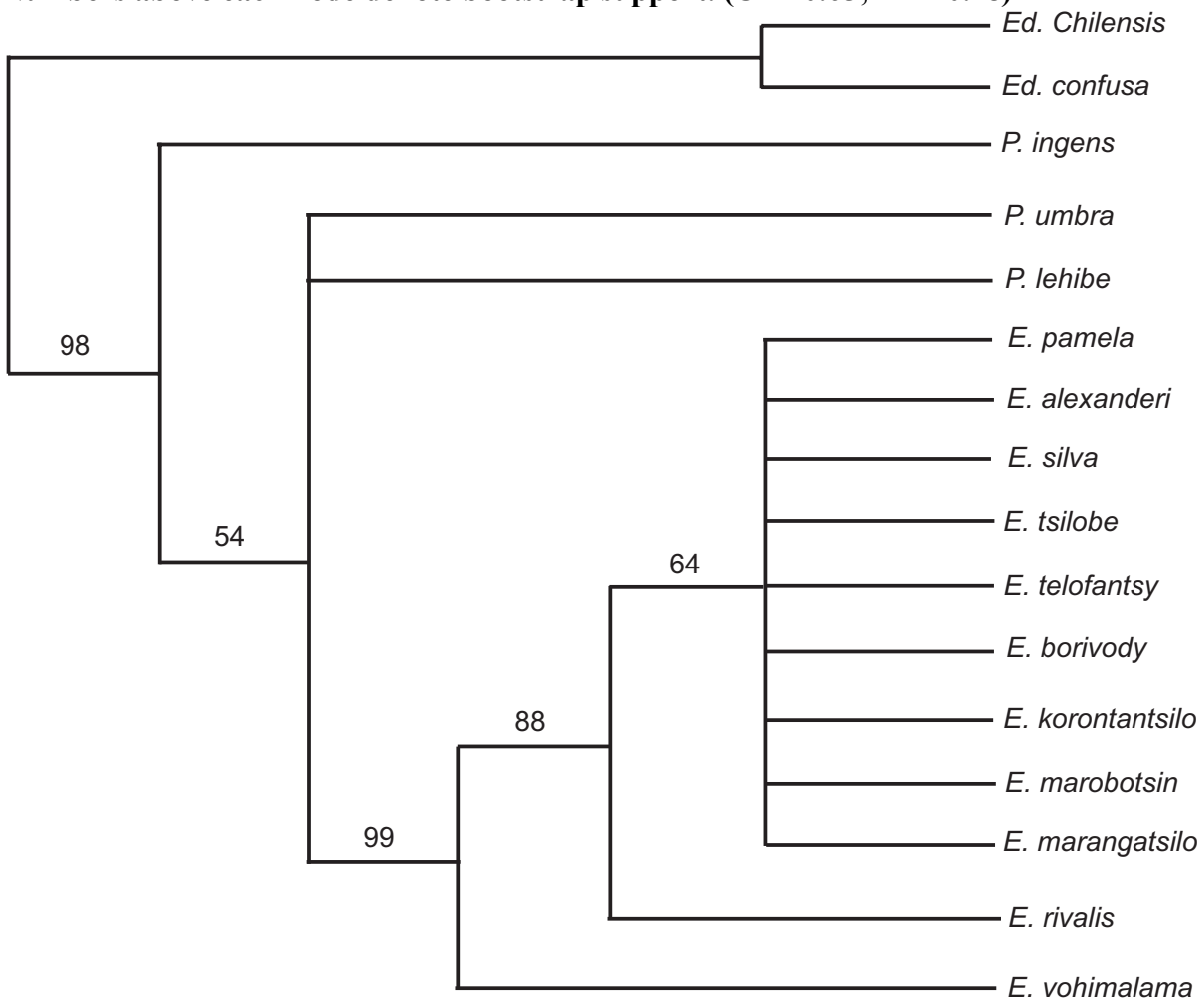


Figure 152: Bayesian inference tree based on morphological characters of all life stages. Numbers above each node denote posterior probabilities.

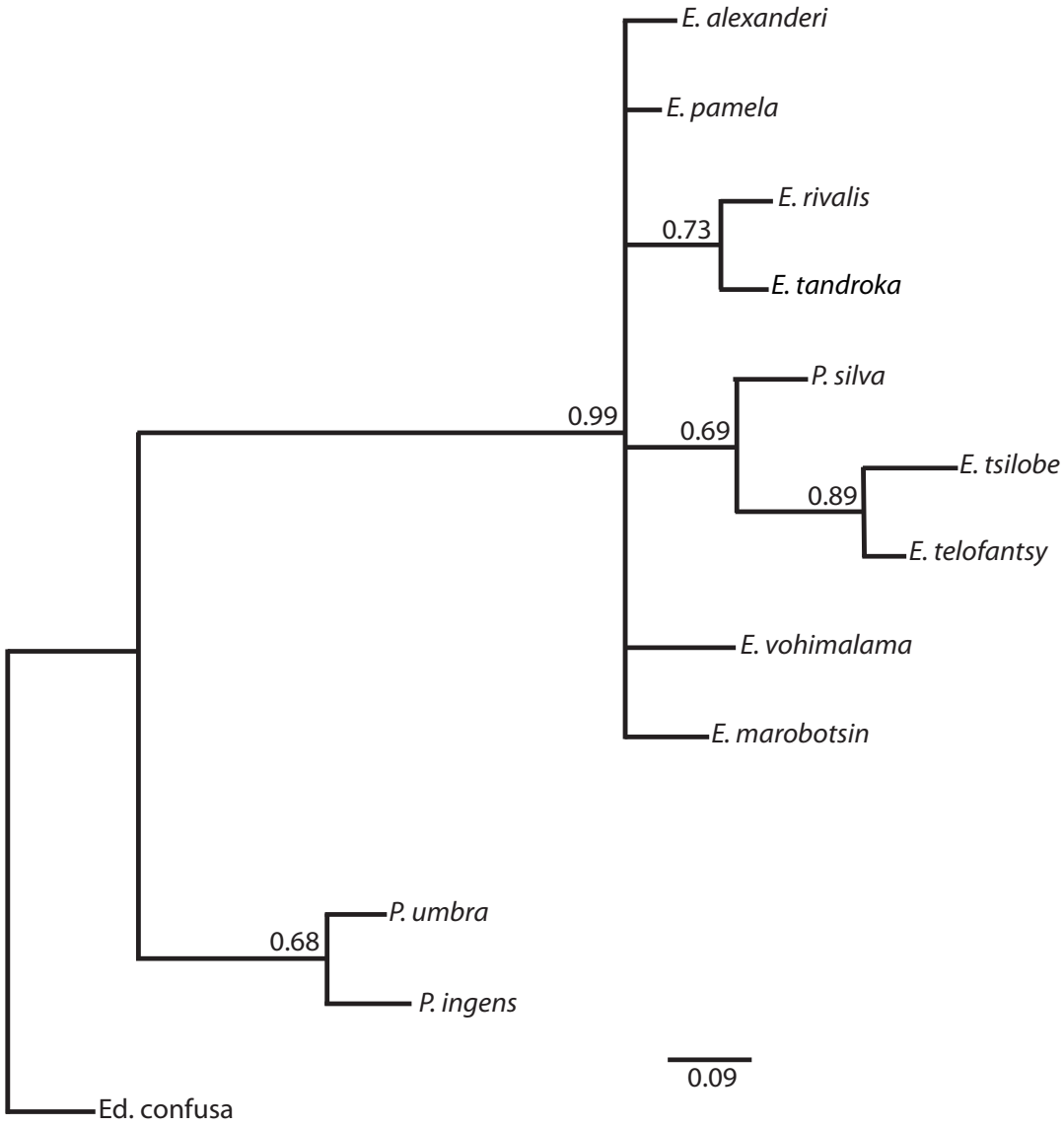


Figure 153: Bayesian inference tree based on nuclear gene BZF2. Numbers above each node denote posterior probabilities.

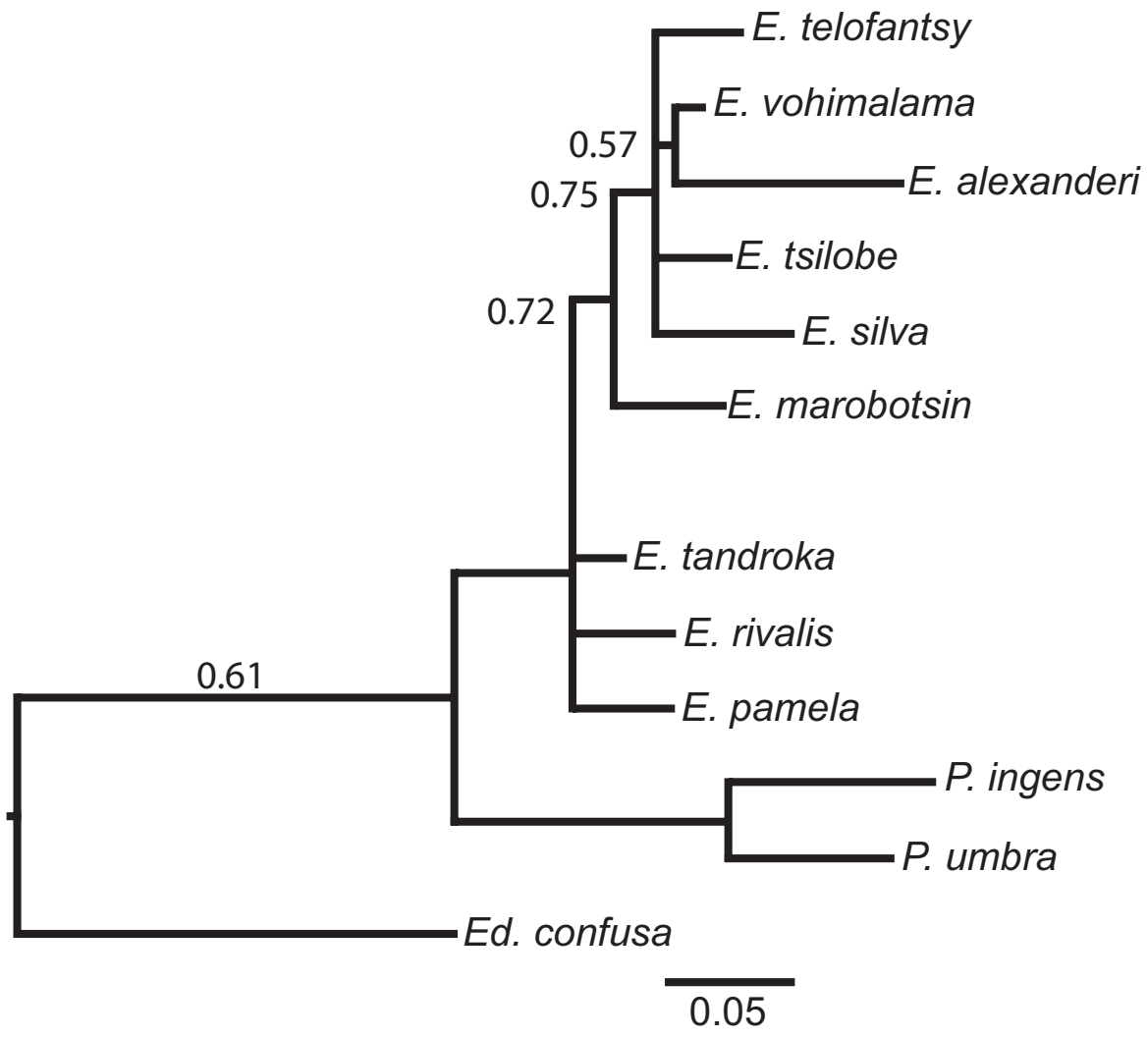


Figure 154: Bayesian inference tree based on mitochondrial gene ND2. Numbers above each node denote posterior probabilities.

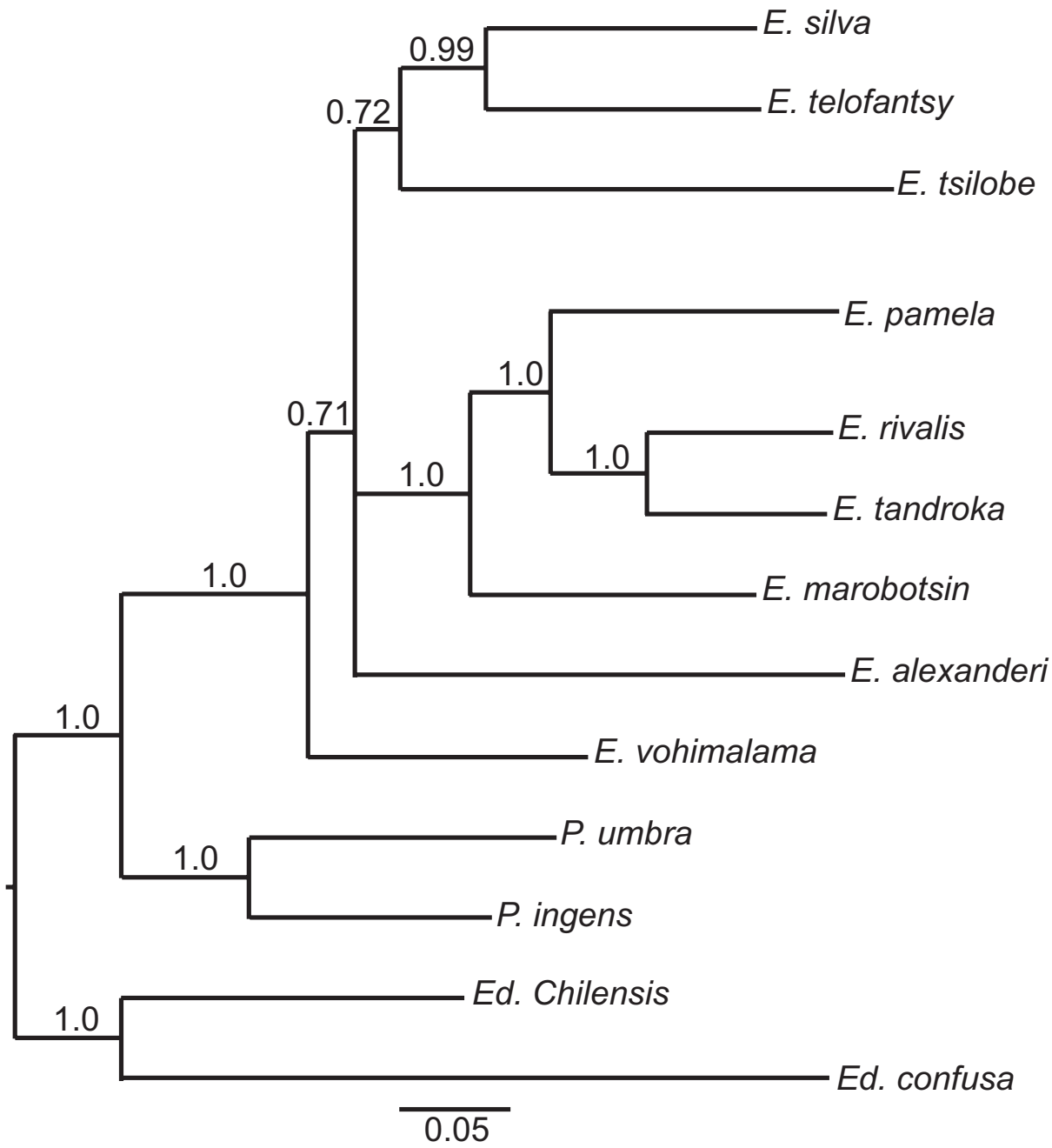


Fig. 155: Bayesian inference tree based on combined morphological and molecular data. Numbers above each node denote posterior probabilities.

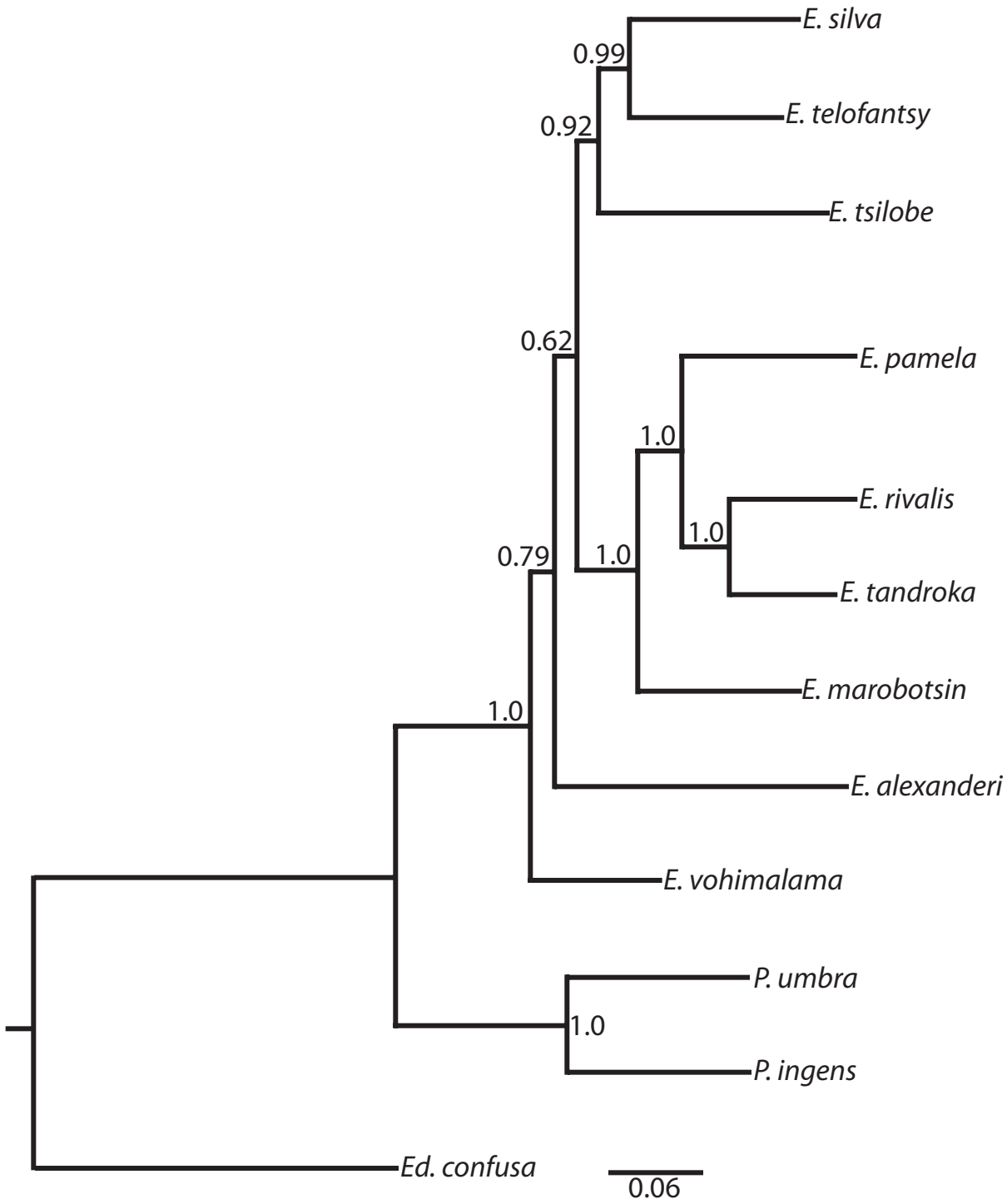


Table 2: Distribution of species (all life stages) across collection sites

	Ambohitantely	Mantadia	Analamazoatra	Vohimana	Sahatondra	Anosibe An'ala/Tolingina R.	Anosibe An'ala/Little Anosibe	Ranomafana	Isaka, nr. Ranomafana	Andringitra	Ambolavao/Jomando R.	Ambalavao/Vakona R.	Ambalavao/Ambaravarandanitra Mt.	Ambalavao/nr. Antanifotsy	Mangabe/Maroantsetra	Ambodivohangy/Maroantsetra	Ambohitsitondroina/Maroantsetra/ Mahalevona	N. of Ivontaka/Antanambe	Ambatobaona/Manjakandriana	Col de Tapia/Antsirabe	Soaindrana Plateau	Rousettes Forest Station/Montagne d''Ambre	Perinet/Andasibe	Mt. Tsaratanana	Manjakatompo Forest Station	Route Anosibe-Toamasina	Tsiafajavona Mountain	Isaka, Fort Dauphine	Androkabe, Fort Dauphine
P. hova																								X					
P. ingens				X						X	X		X											X					
P. umbra	X	X	X	X	X			X															X						
P. lehibe										X			X																
P. ratsipika															X	X				X									
Species J																				X									
E. alexanderi		X	X	X	X	X	X			X	X		X	X				X							X				
E. rivalis	X	X	X					X				X	X		X	X		X					X						
E. robinsoni																										X			
E. tandroka	X	X	X																										
E. pamela	X	X				X		X		X					X								X						
E. silva	X							X		X	X									X					X				
E. tsilobe	X	X		X	X								X																
E. fantsona				X																									
E. marobotsin	X		X	X												X	X					X							
E. vohimalama				X							X																		
E. telofantsy	X									X	X								X	X									
E. botsimpatsy										X				X	X						X								
E. korontantsilo								X		X	X			X					X										
E. marangistilo													X																
E. ratsilo																	X												
E. borivody										X	X				X						X						X		
Species K																X													
Species L																												X	
Species M											X																		
Species N																													X
Species O										X																			
Species P																						X							

Table 3: Distribution of species (all life stages) across elevations

	< 100m	300m	400m	500m	600m	700m	800m	900m	1000m	1100m	1200m	1300m	1400m	1500m	1600m	1700m
P. hova						data unknown										
P. ingens						X										X
P. lehibe																X
P. umbra		X			X	X		X	X					X		
P. ratsipika						data unknown										
Species J																
E. alexanderi		X			X	X			X							X
E. rivalis								X	X						X	
E. robinsoni						data unknown										
E. tandroka								X	X							
E. pamela					X			X	X					X		
E. silva								X						X		X
E. tsilobe						X		X	X					X		
E. pamela																X
E. fantsona						X										
E. marobotsin									X					X		
E vohimalama						X										
E telofantsy														X		
E botsimpatsy																X
E korontantsilo																X
E. marangistilo											X					
E. ratsilo	X															
E. borivody	X															X
Species K						data unknown										
Species L						data uknown										
Species M																X
Species N						data uknown										
Species O																X
Species P									X							

Table 4: Distribution of species (immature life stages) across habitat types

	Mantadia/Cave	Ambohitantely/Below Falls	Ranomafana/Waterworks	Ambohitantely/Above Falls	Mantadia/Swimming hole rocks	Analamazoatra	Mantadia/Sacre Roa	Ranomafana/Roadside streams	Vohimana/Tsat sahina	Vohimana 2	Vohimana 3	Vohimana 4	Analamazaotra	Anosibe Kely	Anosibe an'Ala/Tolingina R.	Vohimana/Sahatandra Trib	Andringitra/Riambavy	Andringitra/Riamdahy	Ranomafana/stream in park	Mantadia/Pika 14	Andringitra/trib	Andringitra/samll falls	Anosibe an'Ala/Mamevo R.	Vohimana/Iasina River	Vohimana 1	Andringitra/Jomando R.	Ranomafana/Navono
	splash zone			madiculous habitat															small stream				large stream				
P. hova									data unknown																		
P. ingens									X		X						X	X				X		X			
P. lehibe																									X		
P. umbra	X			X			X			X		X	X		X	X											
Species J									data unknown																		
E. alexanderi	X						X				X	X		X	X	X	X	X			X		X	X	X		
E. rivalis					X	X	X						X							X							
E. robinsoni									data unknown																		
E tandroka	X				X								X														
E. pamela	X	X		X	X		X	X							X		X	X	X			X					
E. silva		X						X									X	X			X	X					
E. tsilobe	X	X		X			X				X	X	X														
E. pamela		X																									
E. marobotsin	X	X				X																					
E. vohimalama											X																
E. telofantsy				X													X	X								X	
E. botsimpatsy									data unknown																		
E. korontantsilo																	X									X	
E. marangistilo									data unknown																		
E. ratsilo									data unknown																		
E. borivody																	X	X									
Species K									data unknown																		
Species L									data unknown																		
Species M																											X
Species N									data unknown																		
Species O									data unknown																		
Species P									data unknown																		

Figure 156: Map of Madagascar with provinces and associated capital cities

Figures 157-162. Collecting sites. 157-158, Andringitra National Park (January 2007). 159, Special Reserve Ambohitantely (October 2004). 160-161, Mantadia National Park (October 2004). 162, Anosibe an'Ala (June 2006) 163, Vohimana Special Reserve (June 2006).

Figure 164. Map of ecological zones of Madagascar with historical and recent collections.

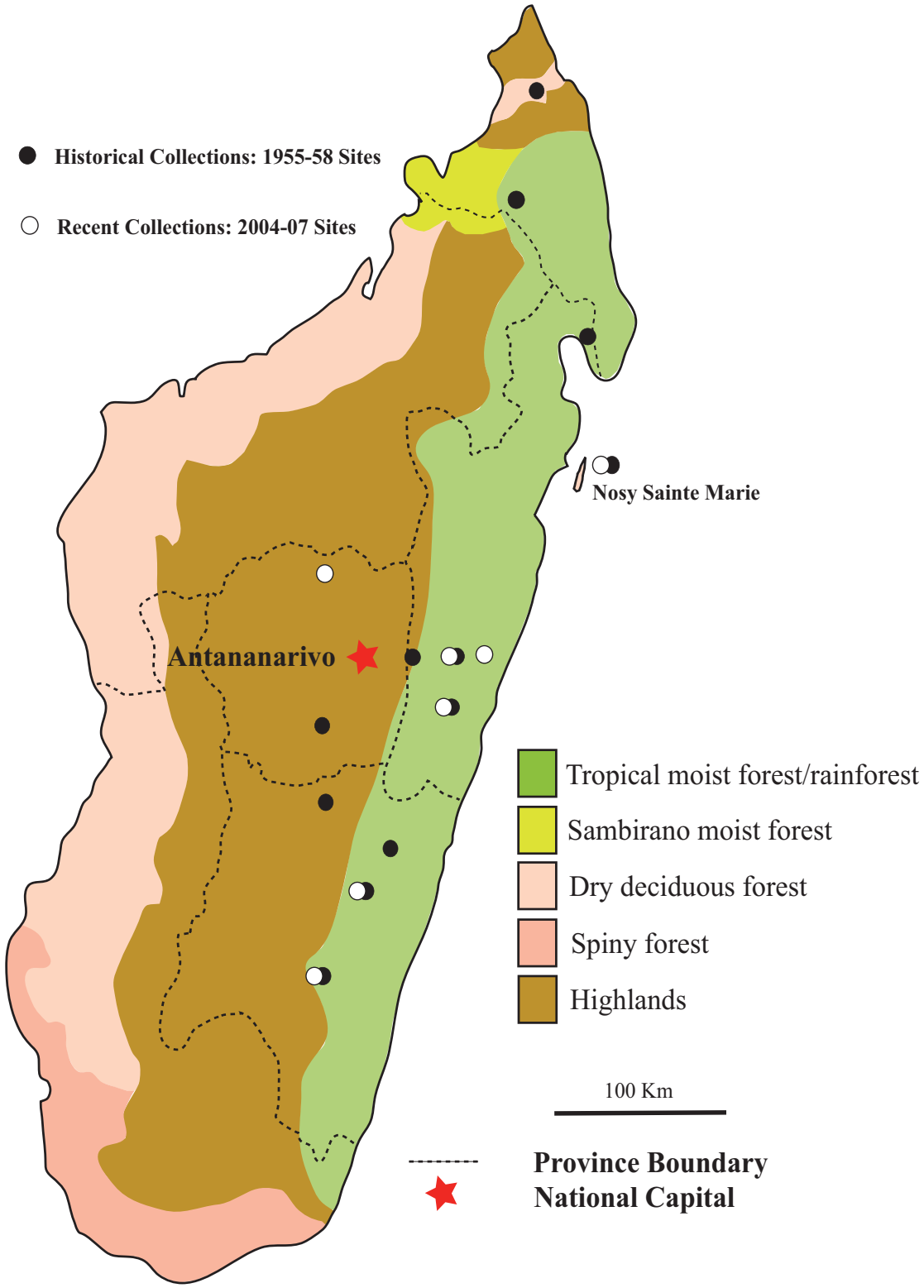


Figure 165. Distribution Map of *Paulianina*

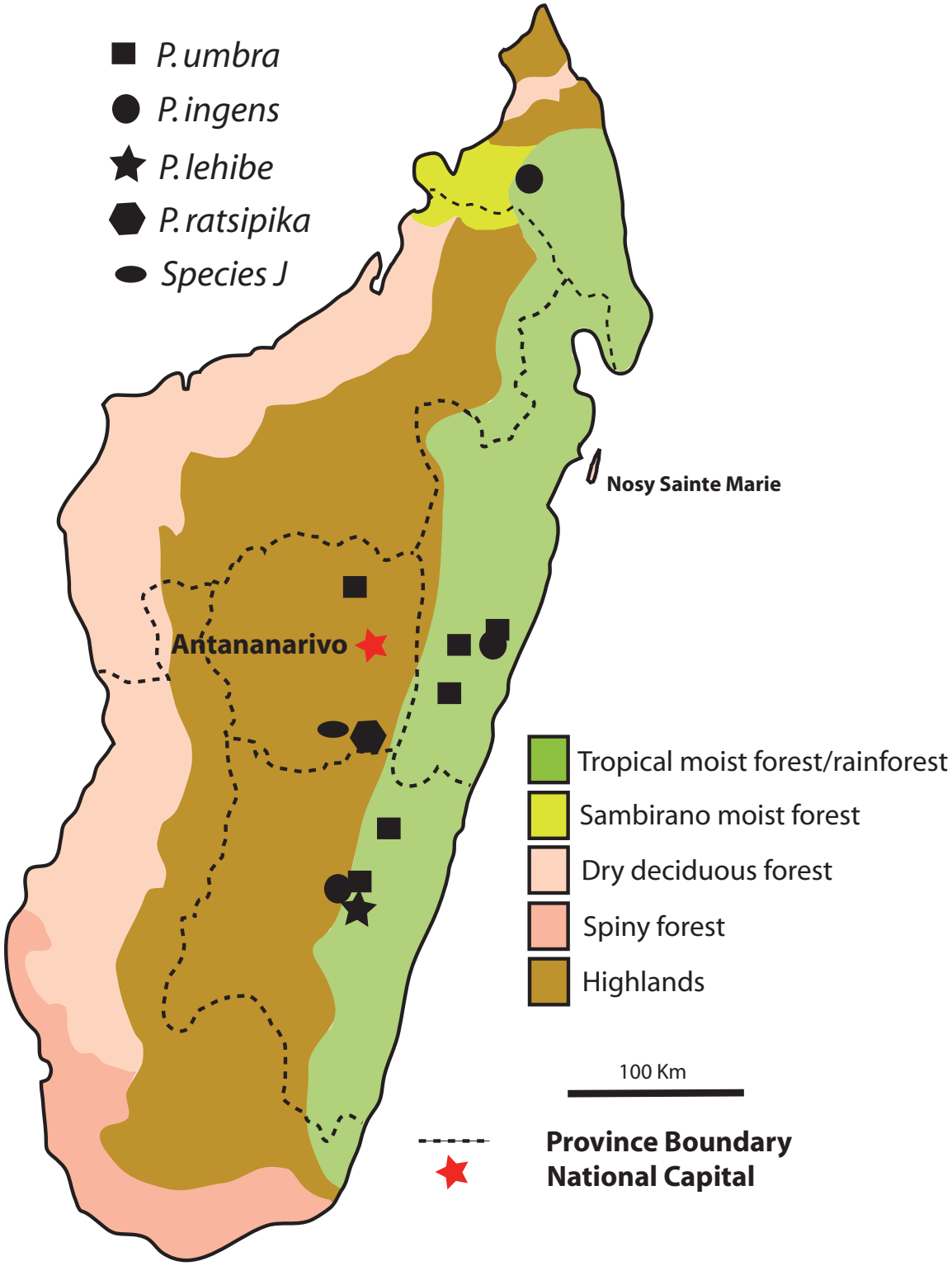


Figure 166. Distribution map *E. alexanderi*, *E. rivalis,* and *E. pamela*.

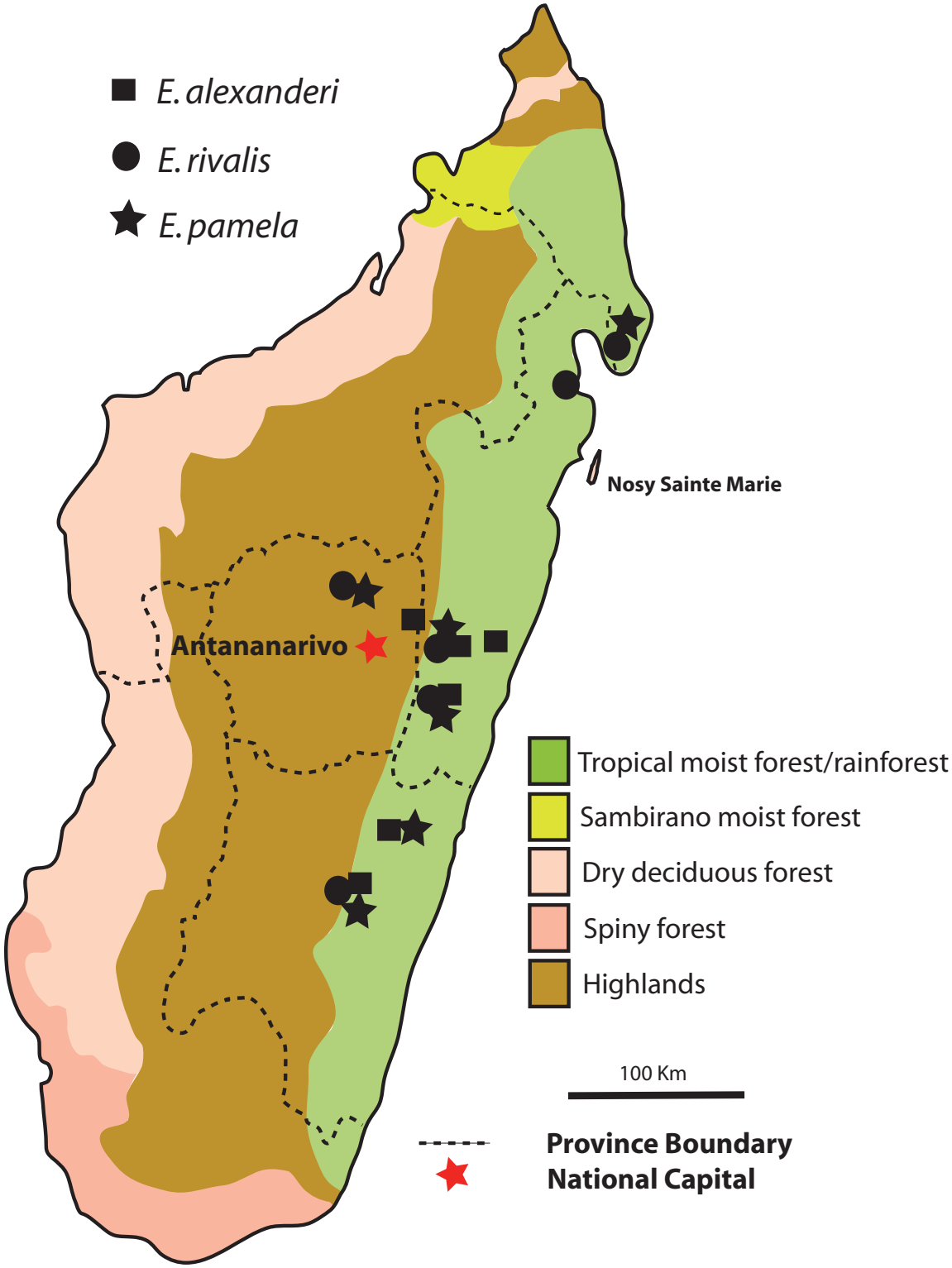


Figure 167. Ditribution Map for *E. silva*, *E. tsilobe*, and *E. telofantsy*.

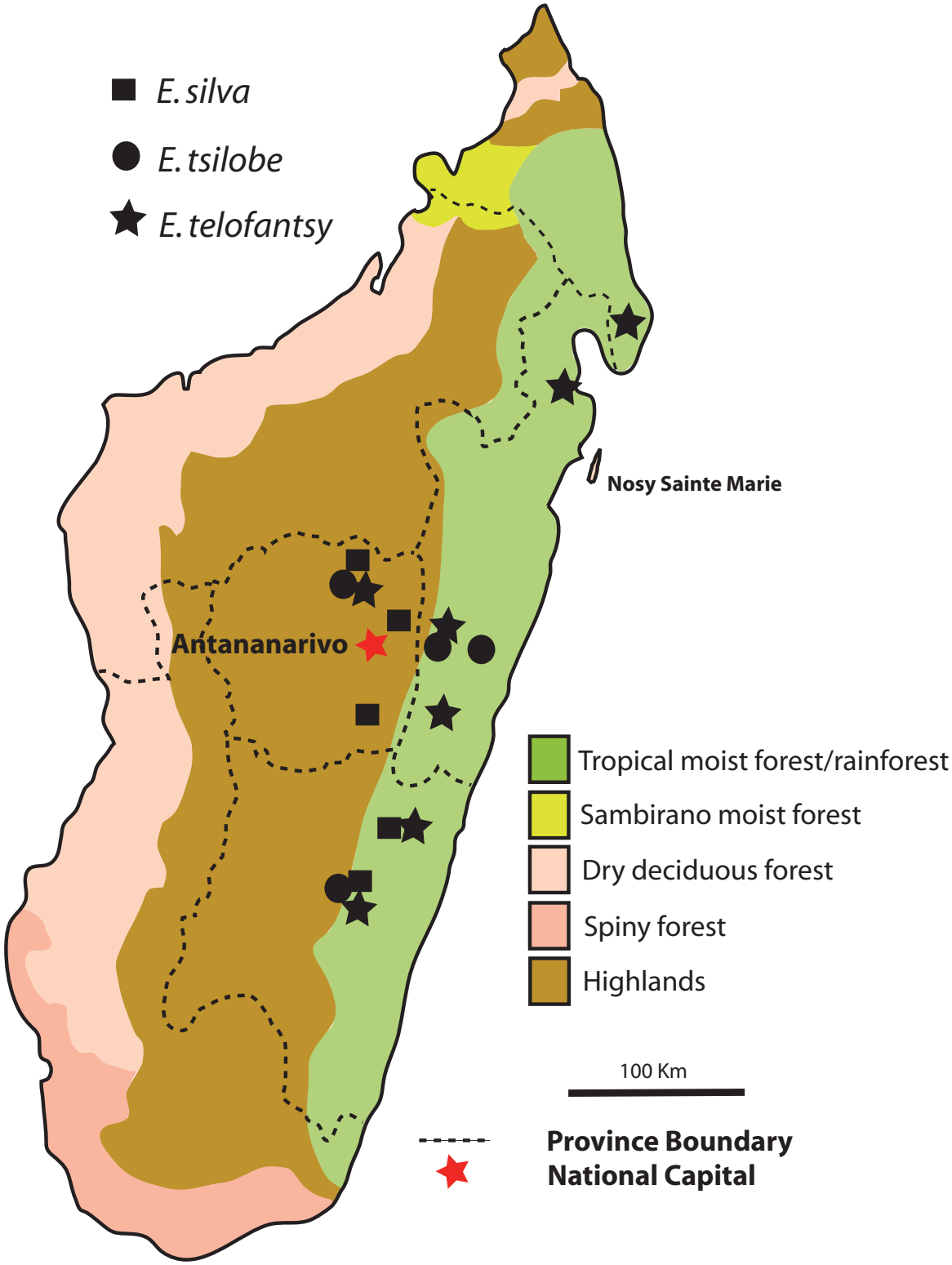


Figure 168. Distribution map for *E marangitsilo*, *E. ratsilo*, and *E. borivody*.

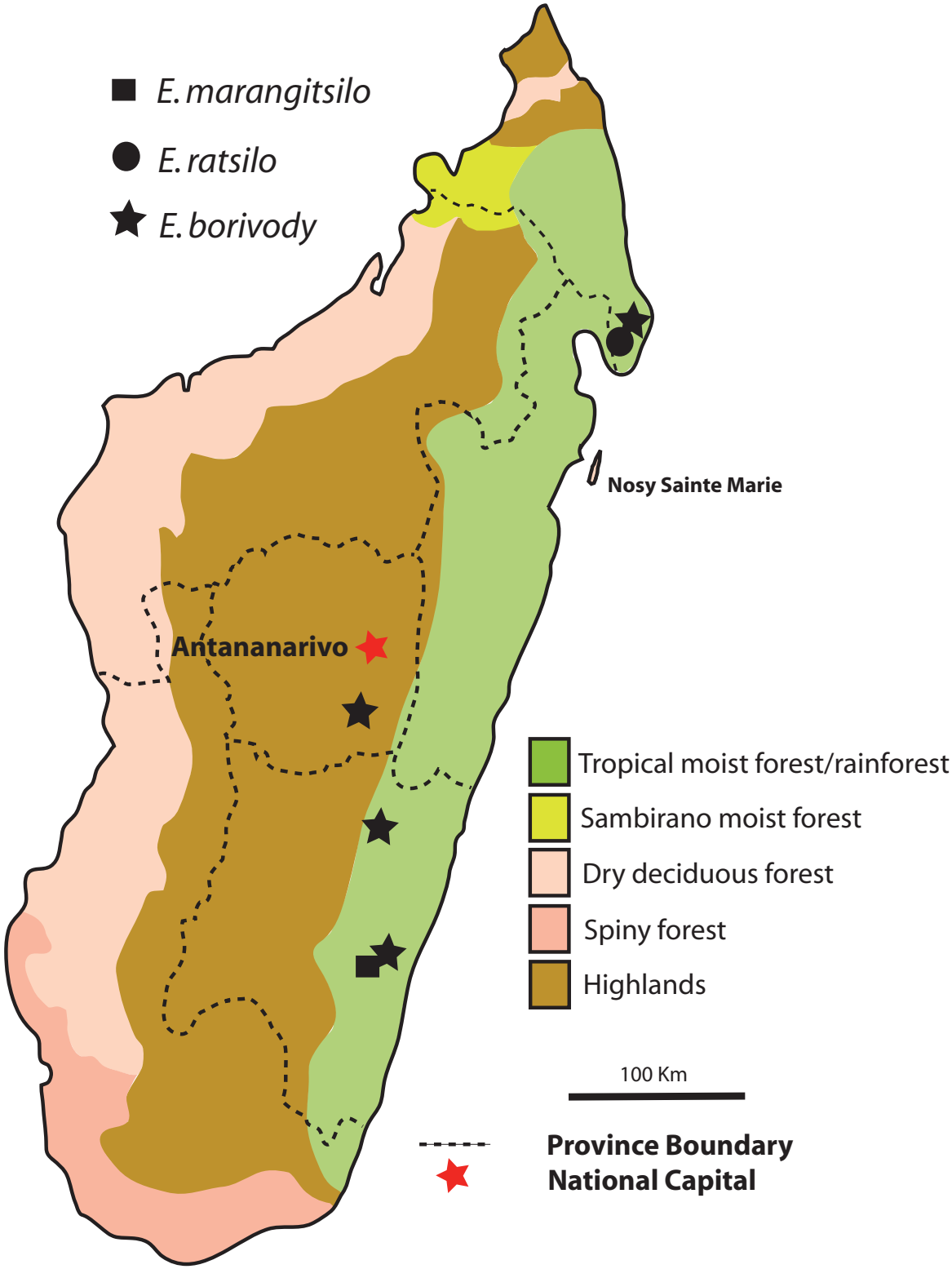


Figure 169. Distribution map for *E. tandroka*, *E. fantsona*, and *E. marobotsin*.

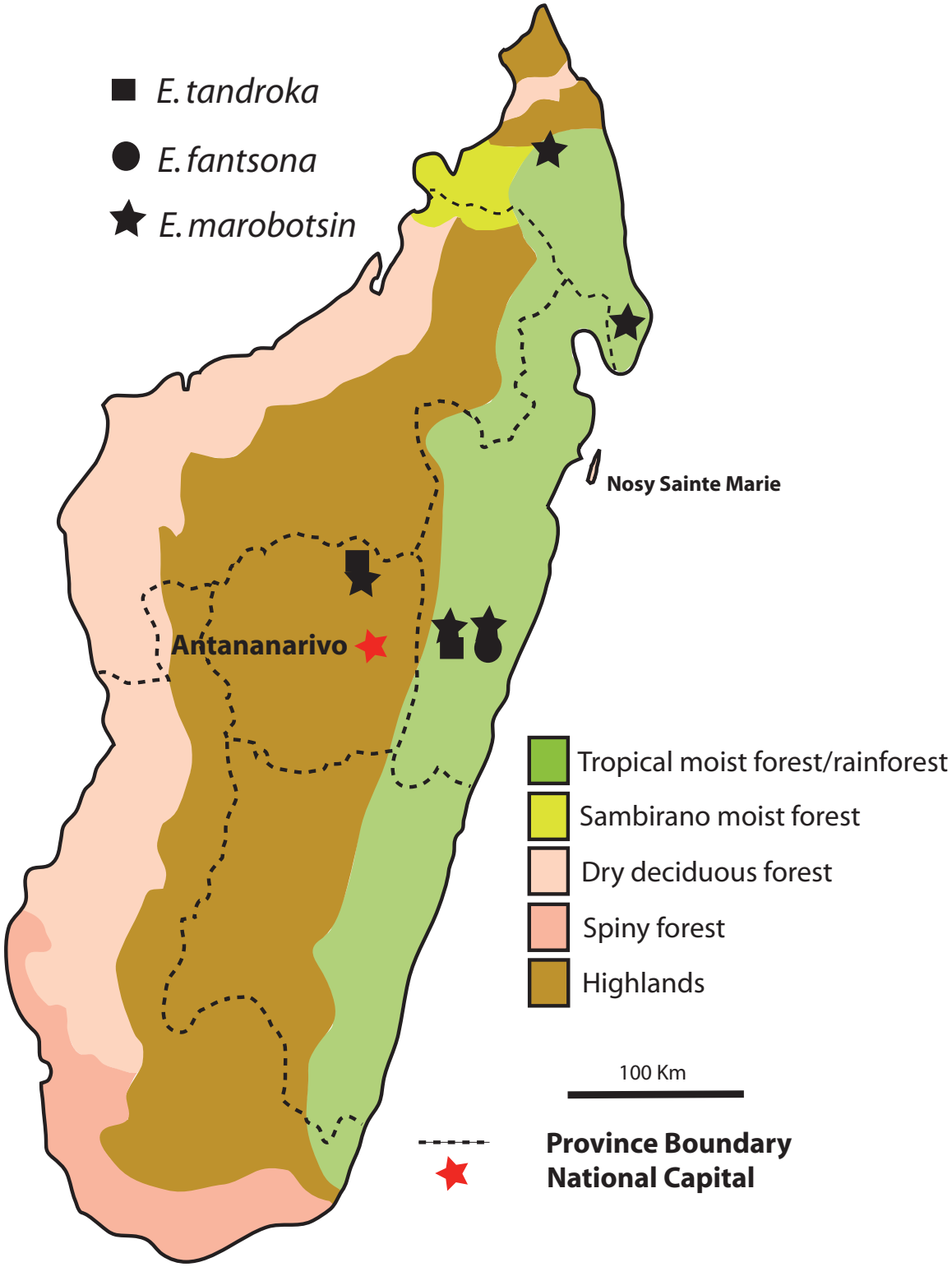
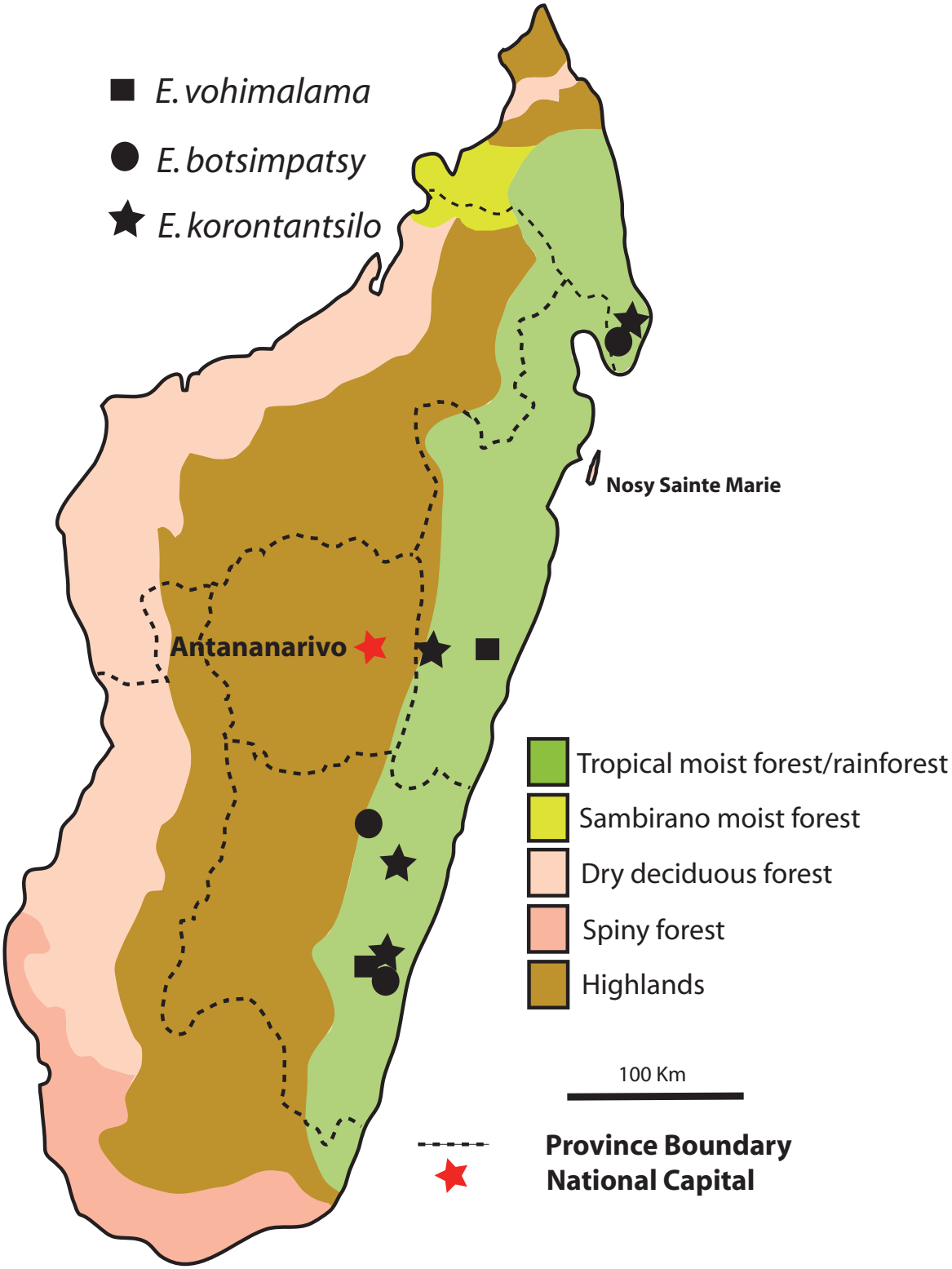


Figure 170. Distribution map for *E. vohimalama*, *E. botsimpatsy*, and *E. korontantsilo*.



LITERATURE CITED

Alexander, C. P. 1952. A new genus and species of net-winged midge from Madagascar (Diptera: Blepharoceridae). *Memoires de L'institute Scientifique de Madagascar* (E) I, fasc. **1**: 227-230**.**

Alexander, C. P. 1953. Undescribed species of Nematocerous Diptera. Part III. *Bulletin of the Brooklyn Entomological Society* **XLVIII (4)**: 97-103.

Alexander, C. P. 1958. Geographical distribution of the net-winged midges (Blephariceridae, Diptera). *Proceedings of the 10th International Congress of Entomology* **1**: 813-828.

Alexander, C. P. 1963. The crane flies of Madagascar in the Natural History Museum, Basel, collected by Fred Keiser. *Verhandl. Naturf. Ges. Basel.* **74** (2): 181-229.

Alverson, A. J. and G. W. Courtney. 2002. Temporal patterns of diatom ingestion by larval net-winged midges (Diptera: Blephariceridae: *Blepharicera*). *Freshwater Biology* **47**: 2087-2097.

Alverson, A. J., G. W. Courtney, and M. R. Luttenton. 2001. Niche overlap of sympatric *Blepharicera* larvae (Diptera: Blephariceridae) from the southern Appalachian Mountains. *Journal of the North American Benthological Society* **20**: 564-581.

Anderson, N. H. 1992. Influence of disturbance on insect communities in Pacific Northwest streams. *Hydrobiolobia* **248**: 79-82.

Arens, W. 1995. Structure and evolution of spiracular gills in pupae of net-winged midges (Nematocera; Blephariceridae). Part I. *Paulianina* and *Edwardsina* (subfamily Edwardsininae). *Canadian Journal of Zoology* **73**: 2318-2342.

Arens, W. 1997. Structure and evolution of spiracular gills in pupae of the Australian species of *Edwardsina* (Insecta: Diptera: Blephariceridae). *Zoologischer Anzeiger* **236**: 231-257.

Arens, W. 1998. Structure and evolution of spiracular gills in pupae of net-winged midges (Nematocera; Blephariceridae): Part III. Gill diversity in *Paulianina* (subfamily Edwardsininae). *Annals of Natal Museum* **39**: 83-114.

Beck R.A., D. W. Burbank, W. J. Sercombe, G. W. Riley, J. K. Barndt, J. R. Berry, J. Afzal, A. M. Khan, H. Jurgen, J. Metje, A. Cheema, N. A. Shafique, R. D. Lawrence and M. A. Khan. 1995. Stratigraphic evidence for an early collision between northwest India and Asia. *Nature* **373**: 55-58.

Bertone, M. A., G. W. Courtney and B. M. Wiegmann. Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. *Systematic Entomology* **33**: 668-687.

Briggs, J. C. 2003. The biogeographic and tectonic history of India. *Journal of Biogeography* **30**: 381-388.

Burney, D. A. 1997. Tropical islands as paleoecological laboratories: gauging the consequences of human arrival. *Human Ecology* **25 (3)**: 437-457.

Courtney, G. W. 1990. Cuticular morphology of larval mountain midges (Diptera: Deuterophlebiidae): implications for the phylogenetic relationships of Nematocera. *Canadian Journal of Zoology* **68**: 556-578.

Courtney, G. W. 1991. Phylogenetic analysis of the Blephariceromorpha, with special reference to mountain midges (Diptera: Deuterophlebiidae). *Systematic Entomology* **16**: 137-172.

Courtney, G. W. 2000a. Family Blephariceridae. In *Contributions to a Manual of Palearctic Diptera. Appendix*. Papp, L. and Darvas, B. (eds.). Budapest: Science Herald. pp 7-30.

Courtney, G. W. 2000b. Revision of the net-winged midges of the genus *Blepharicera* Macquart (Diptera: Blephariceridae) of eastern North America. *The Entomological Society of Washington* **23**: 1-99.

Courtney, G. W. **2003.** Blephariceridae, Net-Winged Midges. In *The Natural History of Madagascar*. Goodman, S.M. and Benstead, J.P. (eds.). Chicago: University of Chicago Press. pp 702-708.

Courtney, G. W. and R. M. Duffield. 2000. Net-winged midges (Diptera: Blephariceridae): A food resource for Brook Trout in montane streams. *Pan-Pacific Entomologist* **76**: 87-94.

Courtney, G. W., R. W. Merritt, K. W. Cummins, M. B. Berg, D. W. Webb and B. A. Foote. 2008. Ecological and distributional data for larval aquatic Diptera. in *An Introduction to the Aquatic Insects of North America. 4th Edition*. R.W. Merritt, K.W. Cummins and M.B. Berg (eds). Dubuque, Iowa: Kendall / Hunt Publishing Co., pp. 747-771.

Craig D. A. 1990. Behavioral Hydrodynamics of cloeon-dipteran larvae (Ephemeroptera, Baetidae). *Journal of the North American Benthological Society* **9 (4)**: 346-357.

Draper, R. 2010. Madagascar's pierced heart. *National Geographic* September Issue.

Edwards, F. W. 1929. Diptera of Patagonia and South Chile, Part II, Fasc. II. *British Museum*, London. Pp. 33-75.

Elouard, J. M. and F. M. Gibon 2003. Ecology of aquatic invertebrates. In *The Natural History of Madagascar*. Goodman, S.M. and Benstead, J.P. (eds). Chicago: University of Chicago Press. pp 511-517.

Frutiger, A. 2002. The function of the suckers of larval net-winged midges (Diptera: Blephariceridae). *Freshwater Biology* **47**: 293-302.

Gibson, R., J. Thompson and D. Higgins. 2009. Clustal X v2.0.23. Published by the author.

Goodman, S. M. and J. P. Benstead (eds). 2003. *The Natural History of Madagascar*. The University of Chicago Press, Chicago, 1709 pp.

Grimaldi D. A., M. S. Engel and P. C. Nascimbene. 2002. Fossiliferous cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates* **3361**: 1-72.

Hogue, C. L. 1986. Recent discoveries in the *Blepharicera tenuipes* group, including description of two new species from Appalachia (Diptera: Blephariceridae). *Contributions in Science*, *Natural History Museum of Los Angeles County* **377**: 1-20.

Hogue, C. L. and I. Bedoya-Ortiz. 1989. The net-winged midge fauna (Diptera: Blephariceridae) of Antioquia Department, Colombia. *Contributions in Science, Natural History Museum of Los Angeles County* **413**: 1-57.

Irwin, M. E., Schlinger, E. I. and F. C. Thompson. 2003. Diptera, True flies. In *The Natural History of Madagascar*. Goodman, S.M. and Benstead, J.P. (eds). Chicago: University of Chicago Press. pp 692-702.

Johns J. A. 1996. The net-winged midges (Diptera: Blephariceridae) of the southeaster United States: phenolgy and ecology. Iowa State University: Masters Thesis. 1-80pp.

Lenat, D. R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water quality ratings. *Journal of the North American Benthological Society* **12**: 279-290.

Lin, C-P. and B. N. Danforth. 2004. How do insect nuclear and mitochondrial substitution patterns differ? Insights from Bayesian analysis of combined datasets. *Molecular Phylogenetics and Evolution* **30**: 686-702.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Oosterbroek, P. and G. W. Courtney. 1995. Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society* **115**: 267-311.

Paulian, R. 1949. Sur la faune des cascades a Nosy Be. *Nature Malgache*. **1**: 31-32.

Paulian, R. 1953. Faune des eaux douces de Madagascar. II. Larves de Blepharoceridae [Dipt.]. *Memoires de L'institute Scientifique de Madagascar*. Serie E, Tome IV: 431-440.

Pommen, G. D. W. and D. A. Craig. 1995. Flow patterns around gills of pupal net-winged midges (Diptera: Blephariceridae): possible implications for respiration. *Canadian Journal of Zoology* **73**: 373-382.

Ronquist, F. and J. P. Huelsenbeck 2003. Mr. Bayes 3: Bayesian phylogentic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

Seward, D., D. Grujie and G. Schreurs. 2004. An insight into the breakup of Gondwana: identifying events through low-temperature thermochronology from the basement rocks of Madagascar. *Tectonics* **23**: C3007.

Stuckenberg, B. R. 1958. Taxonomic and morphological studies on the genus *Paulianina* Alexander (Diptera: Belpharoceridae). *Memoires de L'institute Scientifique de Madagascar* **10**: 97-198.

Swofford, D. L. 2002. *PAUP: Phylogenetic Analysis Using Parsimony.* Version 4.0b10. Sinauer, Sunderland, MA.

Vågen, T-G. 2006. Remote sensing of complex land use change trajectories - a case study from the highland of Madagascar. *Agriculture, Ecosystems, and Environment* **115**: 219-228.

Warren, B. H., D. Strasberg, J. H. Bruggemann, R. P. Prys-Jones and C. Thébaud. **2010.** Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* **26**: 526-538.

Wiegmann, B. M. et al. 2011. Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences* **108 (14)**: 5690-5695.

Wood, D. M. and A. Bokent. 1989. Phylogeny and classification of the Nematocera. *Agricultural Canada Monograph* 32: 1333-1370.

Yoder, A. D. and M. D. Nowak. 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecological Systems* **37**: 405-431.

Zhang, J. and E. D. Lukashevich. 2007. The oldest known net-winged midges (Insecta: diptera: Blephariceridа) from the late Mesozoic of northeast China. *Cretaceous Research* **28**: 302-309.

Zhu, B., W. S. F. Kidd, D. B. Rowley, B. S. Currie and N. Shafique. 2005. Age of initiation of the India-Asia collision in the east-central Himalaya. *Journal of Geology* **113 (3)**: 265-285.

Zwick, P. 1977. Australian Blephariceridae (Diptera). *Australian Journal of Zoology Supplement* **46**: 1-121.

Zwick, P. 1998. Australian net-winged midges of the tribe Apistomyiini (Diptera: Blephariceridae). *Australian Journal of Entomology* **37**: 298-311.

APPENDIX

Figure 1. Matrix of characters and alternate states used in cladisic analysis of *Paulianina* and *Eupaulianina.*

	CharacterNumber																											
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Ed. confusa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ed. chilensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0
P. ingens	1	1	1	1	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1
P. umbra	1	1	1	1	2	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	1
P. lehibe	1	1	1	1	?	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	?	?	?	?	?	?
E. pamela	0	0	1	1	1	1	1	1	1	1	2	2	1	0	1	2	2	0	1	0	2	1	1	1	1	1	0	1
E. alexanderi	0	0	1	1	1	1	1	1	1	1	2	2	1	0	1	2	1	0	1	1	2	0	1	1	1	1	0	1
E. rivalis	0	0	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	2	1	1	1	1	1	0	1
E. silva	0	0	1	1	1	1	1	1	1	1	2	3	1	0	1	3	2	0	1	1	1	0	1	1	0	1	1	1
E. tsilobe	0	0	1	1	1	1	2	1	1	1	2	3	1	0	1	3	1	1	1	1	1	0	1	1	1	1	1	1
E. telofantsy	0	0	1	1	1	1	1	1	1	1	2	3	1	0	1	2	1	1	1	1	2	?	?	?	?	?	?	?
E. borivody	0	0	1	1	?	?	1	1	1	1	2	2	1	1	1	2	1	0	1	1	2	?	?	?	?	?	?	?
E. korontantsilc	0	0	1	1	?	?	1	1	1	1	3	2	1	0	1	2	0	1	0	1	2	?	?	?	?	?	?	?
E. marobotsin	0	0	1	1	?	?	1	1	1	1	3	2	1	0	1	2	1	0	1	1	1	?	?	?	?	?	?	?
E. vohimalama	0	0	1	1	?	?	1	1	1	1	1	1	0	0	0	1	1	0	1	1	1	?	?	?	?	?	?	?
E. marangatsilo	0	0	1	1	?	?	1	1	1	1	3	3	1	0	1	3	0	1	0	1	2	?	?	?	?	?	?	?

Figure 2. Character state distribution across hypothesized parsimony phylogenetic relationships. Numbers above each node denote bootstrap value.

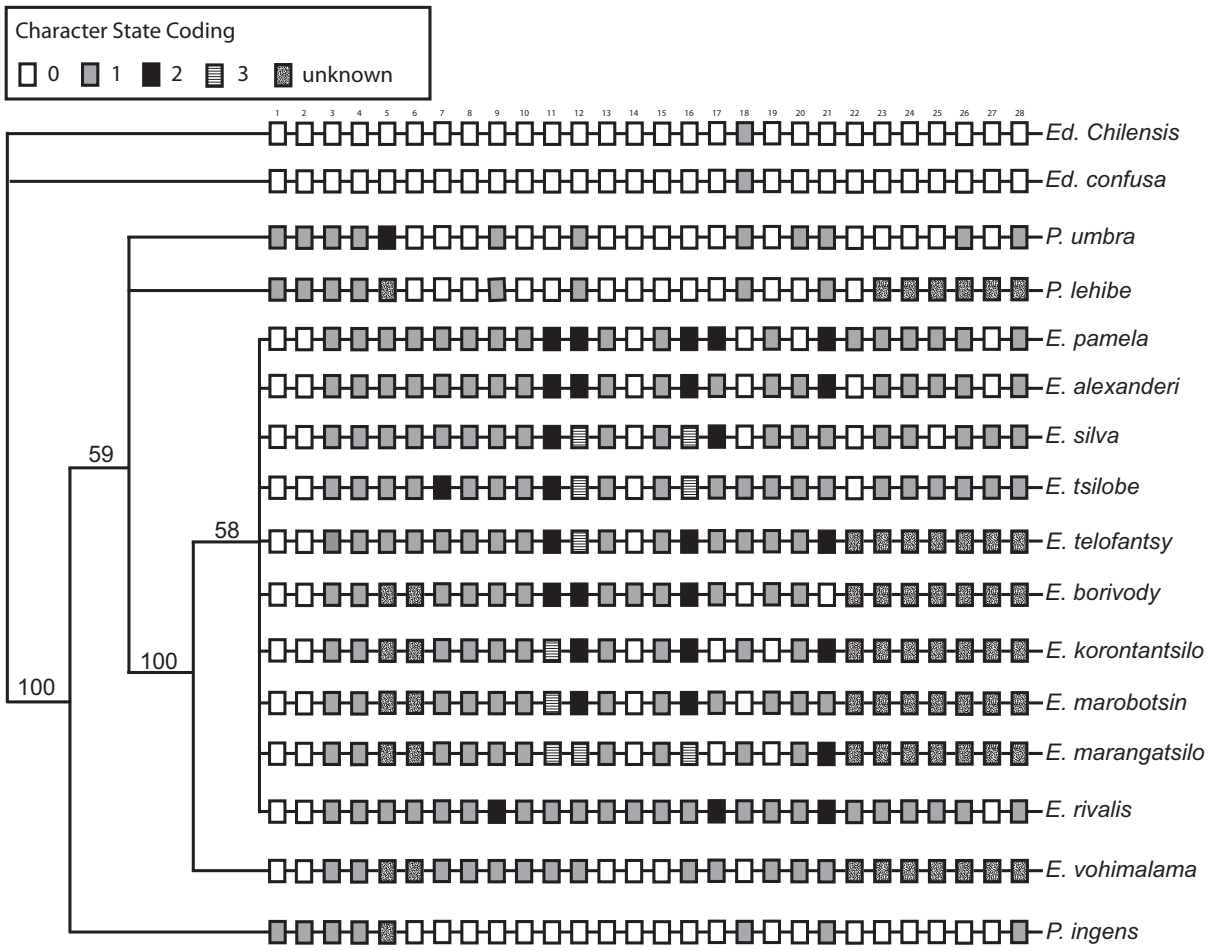


Figure 3. Character state distribution across hypothesized Bayesian phylogenetic relationships. Numbers above each node denote posterior probabilities.

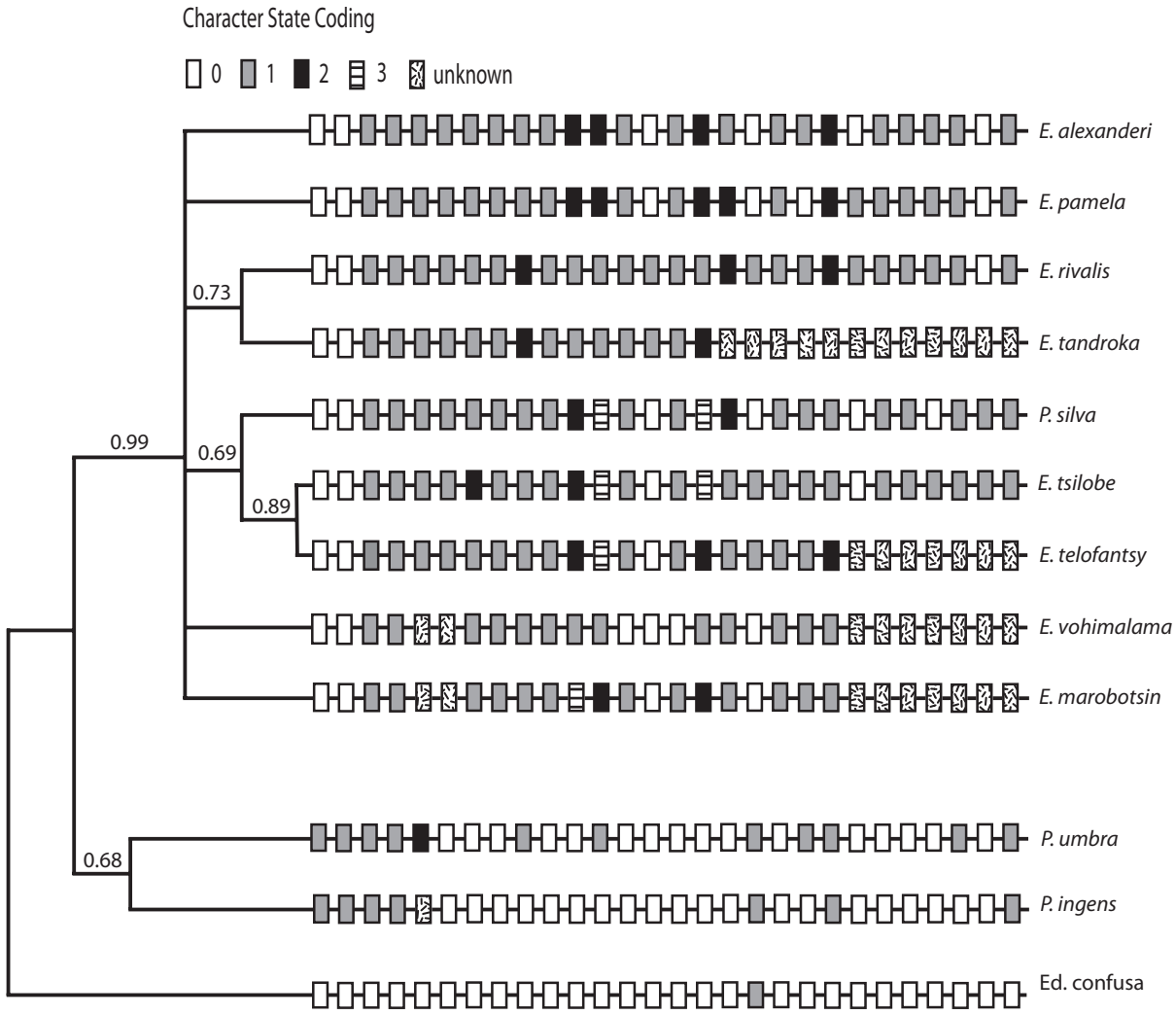


Figure 4. Hypothesized Bayesian phylogenetic relationships between *Paulianina* and *Eupaulianina* larvae and pupae. Numbers above each node denote posterior probabilities.

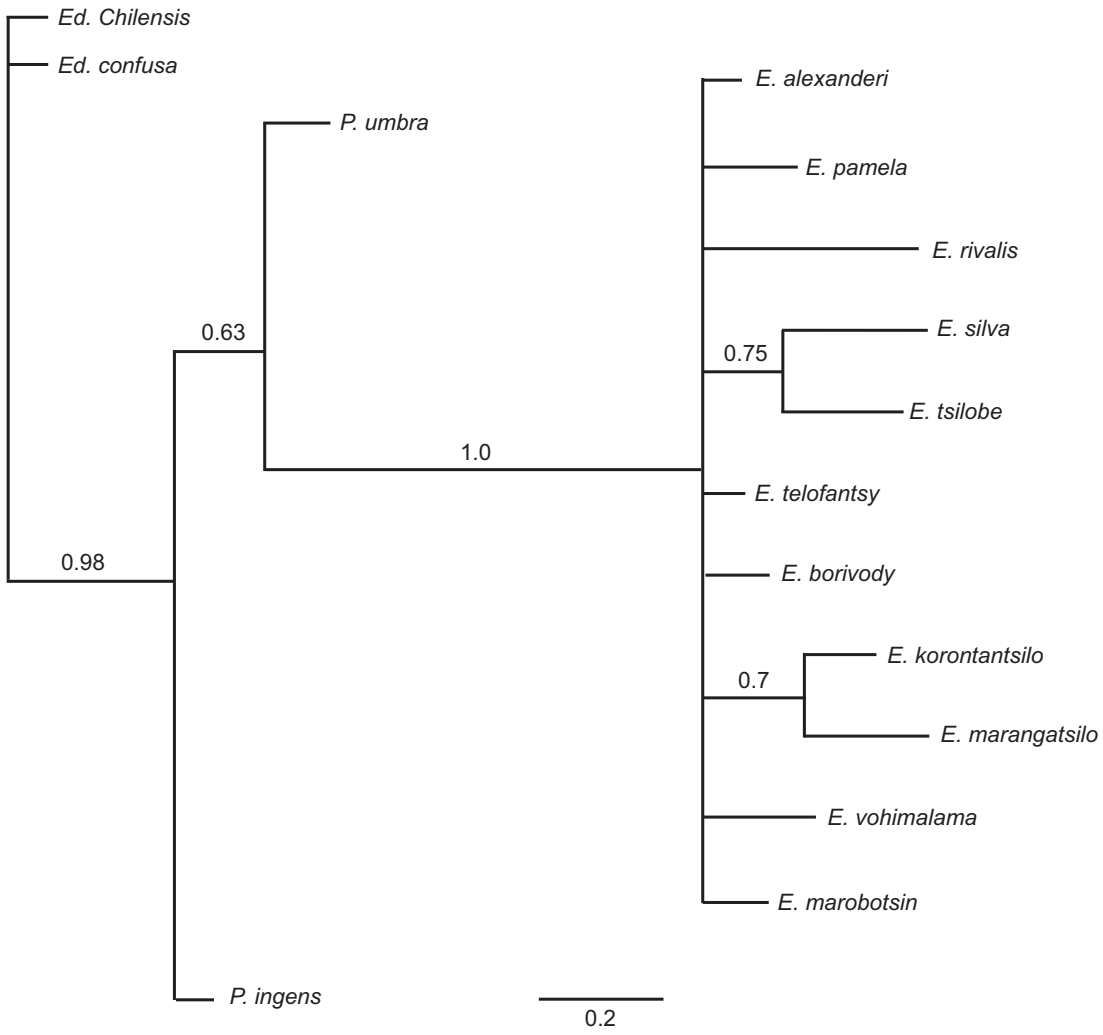
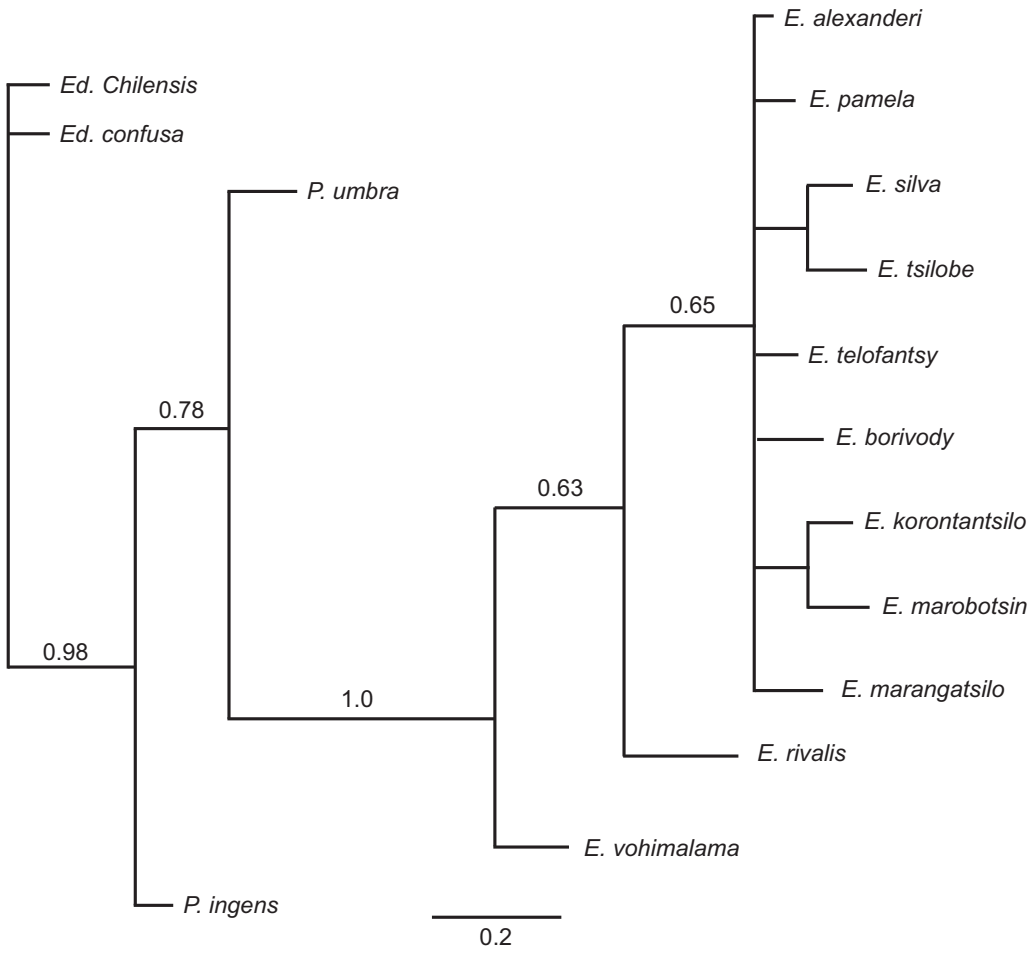


Figure 5. Hypothesized Bayesian phylogenetic relationships between *Paulianina* and *Eupaulianina* larvae and pupae not including pupal papillae characteristics. Numbers above each node denote posterior probabilities.



COLLECTING SITE INFORMATION
(Figs. 157-164)
Data From 2004-2007

Ambohitantely Special Reserve. (Fig. 159) (S18° 11.88’ E47° 16.89’). Ambohitantely is approximately 125 km north of Antanananarivo (Tana) in the Antananarivo Province. It is a small fragment, 5600 has, of the original montane forest ecosystem that once covered the highlands. The reserve has a high diversity, though it is much drier than the eastern rainforests. The single waterfall within the park yielded at least 2 new species.

Analamazaotra Special Reserve. (S18° 55.717’ E48° 25.467'). Located in the Antananarivo Province, this reserve is commonly referred to as Perinet Reserve, The reserve is 810 has and in the same village as Mantadia National Park (see below). There is one small stream in the northwestern part of the reserve, which yielded several species of blephariceridae. The bedrock has a dark coloration, making it difficult to spot specimens.

Andringitra National Park. (Figs. 157-158) (S22° 08.73’ E46° 53.46’). Andringitra NP, which is over 31,000 has, is 47 km south of Ambalavao in the Fianarantsoa Province. It is characterized by steep terrain, and a wide variety of habitats. Collecting was focused on the montane forest next to the Riambavy and Riandavy Falls located on the highest peak on the northern edge of the park. Stuckenberg's original description of the area, based on a 1958 visit, included a colorful image of waterfalls surrounded by rainforest rich in lemurs and insects (Stuckenberg 2002, personal communication to G.W Courtney). The latter included a still unnamed blephariceridae thought to represent a new genus of Apisotmyiinae. One of the goals of a 2007 visit to the site was to recollect the latter and any other resident blephariceridae.

Anosibe An 'Ala. (Fig. 162) **(**S19˚ 25.917' E48˚ 12.282'). Anosibe an'Ala is a small town 70km south of Moramanga. The town is set among remnant rainforest, most of which has been cleared to support rice-based agriculture. Despite this, the area includes several steams that harbor blephariceridsTolingina River is a step-like bedrock falls on the west side of the road where larvae and pupae can be abundant. The Mamevo River (a sacred river to local people) flows along the east side of the road and is a large, deep river with large boulders and fast rapids. Few larvae have been collected at this site. Anosibe Kely, a medium sized stream approximately 3km east of town, flows over a long bedrock ramp and boulders, thereby creating a long waterfall.

Ile St. Marie. (S16˚50.767' E49˚57.067'). This is a small island off the northeastern coast south of Mananara in the Toamasina Province. Previous Collection records from 1958-59 indicated that blephariceridsoccurred in streams that flowed directly into the Indian Ocean. In 2007, two streams approximately 10 km north of Ambodifotatra were investigated, but did not yield any specimens.

Mantadia National Park. (Figs. 160-161) This 17,000ha park is 145km east of Tana in the Antananarivo Province. The park system consists of two protected areas, Special Reserve Analamazaotra (Perinet) and Mantadia National Park. Within Mantadia proper, collecting was done at Peka 14 (S18°47.917' E48°25.717'), a site with a small shaded rocky stream. Sacre Roa (S18°49.717' E48°26.417') consists of a large bedrock ramp with water flowing in sheets into a stream consisting of large cobbles with periodic pools. Collecting was done at a waterfall flowing into a deep pool, as well as inside a small cave (1m X 3m) near the natural swimming pool (S18° 49.983' E48° 26.233'), which had a thin film of

flowing water on the walls. Along with the National Park and Reserve, there is a new park, called Parc Mitsinjo, established by local guides. One small stream was investigated, but did not yield any specimens.

Ranomafana National Park. (S21° 15.86' E47° 24.69'). Ranomafana National Park is one of the largest and well-established parks in the country. Part of this is due to the presence of ValBio Research Center, located along the road between the village and the park entrance. A waterfall, known as Sacre Roa, is located about a 45 min hike into the park. Collecting was done from the waterfall downstream to the Namorona River below ValBio. Collecting was also done at the numerous small streams and falls along the road from ValBio to Ranomafana Village. The village Waterworks, located next to the ANGAP [Need to define acronym?] office, yielded numerous specimens as well.

Vohimana Reserve. (Fig. 163) (S18° 56.39' E48° 30.99'). Vohimana "L'homme et l'environment" (MET) is in Vohimana-Ambavahasina (near Beforona) 12 km east of Andasibe. The reserve is in natural forest with numerous streams. Iasina River, next to the reception center, is accessible down a steep, nearly vertical, drop-off. The river is large, deep, and accented by large bedrock boulders. Within the reserve, the Vohimana River runs past the Relaise du Naturalists. Collecting was done along various parts of the river, as well as Tsat sahina, a tributary of the Vohimana River. Another site was located 5km northwest along the railroad that runs next to the reserve. An unnamed tributary flows into the north side of the Sahatandra River (originally from Mantadia). Large boulders create rapids at this site. Upstream of this site, the Sahatandra River is slow moving and not suitable for collecting.

CHAPTER 3: GENERAL CONCLUSIONS

Despite several extensive collection trips over the last six decades (Paulian 1949; Stuckenberg 1958) little was known about the diversity of blephariceridsa unique aquatic group of Madagascar. The purpose of this monograph was to provide the most thorough summary of currently known information about the blephariceridsof the island. An additional goal was to examine the phylogenetic and biogeographic relationships between and within the two genera, *Paulianina* and *Eupaulianina*, present on the island. These analyses were supplemented by a recent collecting expedition (Sam, 2004-2007).

This study complements Stuckenberg's (1958) comprehensive study Malagasy blepariceridsby continuing to document the diversity of all species currently known from the country, increasing the number of described species from eight to twenty-seven. Complete descriptions for each species are supplemented with light micrographs, scanning electron micrographs and illustrations. Dichotomous keys are provided for all life stages, except adult females. This revision is the most complete documentation of Malagasy species. The phylogenetic analysis included both morphological and molecular characters. There has been speculation about the relationship between *Paulianina* and *Eupaulianina*. While the morphological analysis provided new information regarding relationships, the DNA sequencing yielded a large set of characters. The two genera were confirmed to be monophyletic based on the molecular analysis as well as the combined morphological-molecular analysis. Relationships within *Eupaulianina* are still partially speculative, in part because there were a limited number of specimens suitable for DNA extraction. Future collection of new specimens appropriate for molecular analaysis would further elucidate these relationships.

Many species such as *P. umbra*, *E. alexanderi*, *E. pamela*, *E. marobotsin* and *E. tsilobe* are collected across a wide number of locations on the island, while several (*P. lehibe* and *E. vohimalama*), are located within a very limited range of habitat. However, the vast majority of these limited range species are based on single specimens, requiring further collection to fully determine the presense of the species. Several species that were collected in 1958 were not collected in the recent expeditions (Species L-Species P), indicating that these species might possibly be extinct due to habitat destruction.

Although Malagasy blephariceridsdisplay the common ecological habits of the family, much is still unknown about their ecology in terms of number of generations, common predators, major competitors, etc. They are assumed to be multivoltine, however, this, and other ecological data has not been completely documented because of collectiong limitations, including the time allotted for collections as well as adverse weather conditions that are common during the summer rainy months.

The ongoing destruction of suitable habitat across the island has created an ecological emergency, requiring the urgent documentation of the island's biodiversity. Since the collection expedition of 2007, the Ambotavy pipeline construction has destroyed the habitat that was the only known location of *E. fantsona* within the eastern rainforest corridor (Draper 2010). Unfortunately, this scenario is the norm, rather then the exception. Future research within this diverse biological hotspot is imperative if we are to effectively preserve and manage this ecosystem in the future.

LITERATURE CITED

Draper, R. 2010. Madagascar's pierced heart. *National Geographic* September Issue.

Paulian, R. 1949. Sur la faune des cascades a Nosy Be. *Nature Malgache* **1**: 31-32.

Stuckenberg, B. R. 1958. Taxonomic and morphological studies on the genus *Paulianina* Alexander (Diptera: Belpharoceridae). *Memoires de L'Institue Scientifique de Madagascar* **10**: 97-198.

ACKNOWLEDGEMENTS

I sincerely thank Dr. Gregory W. Courtney for serving as my major professor over the past several years of my graduate education. I truly appreciate his support in all aspects of my years as a graduate student at Iowa State University. I express my appreciation to Dr. Lynn Clark for her advice, both pertaining to my research as well as my future. I also thank the remaining members of my committee, Dr. Marlin Rice, Dr. Matt O'Neal, and Dr. Timonthy Stewart for their guidance through this process.

So many people as Iowa State University have provided support throughout my graduate years, including all my labmates, Amanda Jacobson, Matt Wihlm, Andrew Fasbender, Matt Petersen, Jess Petersen and Greg Curler. A special thank you to Amanda, for her wonderful support as a friend, as well as her endless patience while teaching me the intricacies of the unknown world of molecular techniques. Also, special note to Andrew, for his assistance with the phylogenetic analysis.

Special thanks to Angela Tague and other members of the Insect Zoo for their friendship and patience, as well as providing the outlet for my love of teaching. Dr. Russell Jurenka also provided the opportunity for teaching, allowing me to be the teaching assistant for Insect Biology for several years. Thank you Kelly Kyle for always knowing what to do when chaos struck in the official aspects of graduate education.

Of special note, many thanks to Dr. Harry T. (Jack) Horner, Tracey Pepper, and all the staff of the Bessey Microscopy Facility at Iowa State University for their patience in teaching me the skills of scanning electron microscopy that were used to take the images used in this dissertation.

Thank you to Dr. Kevin Moulton of the University of Tennessee, Knoxville for allowing me to partipicate in his lab for two weeks while learning molecular techniques. He provided guidance and answered my endless questions. Also, thank you for sequencing the genes in his lab.

Dr. Mike Irwin, Dr. Martin Hauser, and Dr. Frank Parker deserve special recognition for introducing me to Madagascar in my first collecting expedition to the island. Dr. Hauser contiues to be a valuable friend, and provided sound advice and support thoughout this whole process.

I thank all my colleagues in Madagascar, including my associates at the University of Antananarivo, the MICET group, and Mitsinjo Association. Special thanks to the Sam family for their aid in all aspects of the Madagascar expeditions.

Finally, I thank my family. They have provided much emotional support, and without them, this paper would not be possible. A special thank you to my husband, Olivier Sam, who not only provided emotional support, but also became an expert collector of blephariceridsalong the way. My daughter, Nyssa Brown, deserves special note, for her endless patience as Mommy worked many, many hours at the dining room table.